THE DEVELOPMENT OF PLANETARY PROTECTION REQUIREMENTS FOR HUMAN MARS MISSIONS: A HISTORY. J. D. Rummel[1], M. S. Race[2], and G. Kminek[3], [1]East Carolina University, Greenville, NC 27858 USA, rummelj@ecu.edu, [2]SETI Institute, Mountain View, CA 94043, mrace@seti.org, [3]ESA/ESTEC, Noordwijk, The Netherlands, gerhard.kminek@esa.int.

Introduction: Although NASA's preparations for the Apollo lunar missions had only a limited time to consider issues associated with the protection of the Moon from biological contamination and the quarantine of the astronauts returning to Earth, they learned many valuable lessons (both positive and negative) in the process. As such, those efforts represent the baseline of planetary protection preparations for sending humans to Mars. Neither the post-Apollo experience or the Shuttle and other follow-on missions of either the US or Russian human spaceflight programs could add many additional insights to that baseline. Current mission designers have had the intervening four decades for their consideration, and in that time there has been much learned about human-associated microbes, about Mars, and about humans in space that has helped prepare us for a broad spectrum of considerations regarding potential biological contamination in human Mars missions and how to control it.

This paper will review the approaches used in getting this far, and highlight some implications of this history for the future development of planetary protection provisions for human missions to Mars. The role of NASA and ESA's planetary protection offices, and the aegis of COSPAR have been particularly important in this ongoing process.

Shuttle Era Efforts: As the Space Shuttle was developed and eventually flown (1981), NASA was interested in Shuttle-delivered modules as the basis of space activities, and for some those modules were best envisioned as a space station (then TBD). Of significance to the development of planetary protection thinking in this era was the Antaeus Report [1], which identified specific needs for an orbiting space-station associated module that might be dedicated to the quarantine of a Mars sample. The report suggested the use of the smallest available biological test systems that could be emplaced by the Space Shuttle, then under development. The human element was essential for making this concept feasible.

Humans Exploring Space?: With a Presidential announcement of a Moon/Mars destination for NASA (1989), NASA's initial examination of the challenge [2] and other workshops [3] considered the issues of Mars exploration by humans and the planetary protection challenges associated with the then-current thinking about life on Mars. Sometimes the issues were well-considered, and sometimes less well conceived [4]. As it turned out there would be plenty of subsequent chances to continue to hone the effort to understand the challenges and consequences associated with humans and their microbial "load" in an exploration setting.

One of the first attempts to directly involve both human mission planners, developers, and medical personnel in the process of setting planetary protection requirements took place in Pingree Park, Colorado in June 2001 [5]. This workshop began a series of focused discussions among agency and mission-planner that continued in the first half of that decade. An important contribution a short while after was the *Safe on Mars* report of the National Research Council [6], which considered all of the hazards associated with sending humans to Mars, including chemical and biological ones. A consensus effort came together in April 2005 with a comprehensive workshop at the Lunar and Planetary Institute in Houston [7] that involved the life support and habitation personnel from the human space flight community. At that workshop the participants established three principles: 1) avoid forward contamination of Mars or interference with scientific exploration from terrestrially associated microbial contaminants; 2) protect astronauts from harmful contamination from martian life forms; and 3) control back contamination from the spacecraft, astronauts and materials that are returned to Earth. Finally to wrap up the workshops on planetary protection and humans, a joint NASA/ESA workshop was held at ESTEC in The Netherlands in May of 2005 [8]. That workshop included splinter group discussions organized around three main areas with implications for planetary protection on human rated systems:

- Advanced Life Support Systems (ALS);
- Extravehicular Activities (EVA); and
- Operations and Support (OPS).

These splinter-group discussions considered operations and technology concerns, science activities and operations, backward contamination prevention requirements, and the protection of both the human habitat on Mars and the Earth upon crew return. They also identified future research and development needs for ALS, EVA, and Mars robotic missions, including specific precursor mission information necessary to understand and prepare for human support systems and science operations on long duration Mars missions.

COSPAR's Interim Provisions: The results of the various workshops and considerations regarding humans were largely consolidated by the NASA/ESA workshop in 2005. Subsequently, those results were reported to COSPAR in Beijing in 2006, although no action was taken by the Panel on Planetary Protection at that time. The matter was presented and further discussed in 2008 at the COSPAR Assembly in Montréal [10], which resulted in a Panel on Planetary Protection resolution that went forward to the Bureau and COSPAR Council, and was approved by both groups.

As a result, new section was added to the COSPAR Planetary Protection Policy [11] that includes "Principles and Guidelines for Human Missions to Mars," with the following policy statement as a preamble:

> The intent of this planetary protection policy is the same whether a mission to Mars is conducted robotically or with human explorers. Accordingly, planetary protection goals should not be relaxed to accommodate a human mission to Mars. Rather, they become even more directly relevant to such missions—even if specific implementation requirements must differ.

Presently: The current planetary protection policy documents do not stipulate detailed requirements for future human missions to Mars, but rather provide principles and guidelines of what will be needed for implementation of any successful mission and provide the basis to move forward to develop agency-level and eventually mission-level requirements. Appropriate agency-level documents are (will?) embodying the new requirements as they come forward. Such requirements are essential complements to future human missions beyond Earth orbit, and can contribute to technology development and science activities on the Moon as well as to planning for human mission to Mars and use of its resources. As long as there is a possibility that Mars may have indigenous life, or that there are places on Mars where Earth organisms might survive and grow (cf., 12], planetary protection provisions will be an essential part of activities and exploration on Mars.

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SUMMARY OF THE 2005 LIFE SUPPORT AND HABITATION AND PLANETARY PROTECTION WORKSHOP. J.A. Hogan, NASA Ames Research Center, MS239-15, Moffett Field, CA 94035
john.a.hogan@nasa.gov

Introduction: As seen in previous human lunar and Mars surface robotic missions, Planetary Protection (PP) guidelines will serve as a strong driver in the design and operation of human exploration missions to Mars and other solar bodies. Likewise, science objectives such as the search for evidence of past or present extraterrestrial life can also impact mission design. Therefore PP and science requirements for human missions need to be established well in advance of a mission to facilitate the timely and cost-effective design and execution of compliant spacecraft, habitation systems and surface operations.

The PP requirements development process necessitates a thorough knowledge of potential forward and back contaminants, contamination pathways and a current understanding of the biological potential of the solar body. While numerous considerations are involved in PP policy development, there are (at least) three key research and technology development programs that require close consideration and collaboration. First is the human life support program, which is responsible for managing air, water and solid wastes, and providing food and thermal control. Secondly, the extravehicular activity (EVA) program is tasked with developing portable life support systems to enable human mobility, including suits and rovers. EVA activities will generate forward and back contamination potential via human and equipment ingress/egress operations and leakage. Finally, the monitoring and environmental control program is responsible for developing methods that facilitate the monitoring of contaminants relevant to established PP and scientific guidelines. Together, these three areas will strongly interface with each other and will affect, and be affected by, PP considerations.

Workshop Background and Objectives: Because of the lack of discrete PP regulations for proposed human Mars missions, members of these three communities concluded that establishing a dialogue that enabled PP requirements development was necessary. To this end, a workshop entitled the "Life Support & Habitation and Planetary Protection Workshop" was convened at the Center for Advanced Space Studies in Houston, TX on April 27-29, 2005[1]. Participants included representatives from government, private industry and academia. A major objective of the workshop was to initiate communication, understanding, and a working relationship between the life support, monitoring and control, EVA and the PP communities regarding the effect of PP policy development and implementation requirements for future human missions. It was also intended to define top-level PP concerns and issues associated with both forward and back contamination, and determine their likely effects on hardware development and operations for the first human mission to Mars. This included the identification of PP requirements that will be needed to guide future technology development in advance of the first human mission. The workshop was also designed to identify management approaches to reduce the risk of developing systems prior to full definition of PP policies, as well as critical research areas and gaps in science or technology capability. Participants were provided initial assumptions to provide defined boundaries during deliberations.

Summary of Overall Workshop Findings: An array of findings and recommendations were generated during the workshop. The top-level areas included the examination and identification of potential forward and backward contaminants and associated release pathways. Mitigation techniques for both forward and backward contamination were also discussed and identified. Participants identified crucial factors likely to impede hardware and system development with respect to potential PP requirements. Finally, key research and technology needs resulting from PP requirements were identified. Top-level findings include:

- While there is a lack of explicit PP policies and requirements for human missions to Mars, it is possible to outline a conceptual approach and provide preliminary guidelines for planners and designers. The development of more specific guidelines will occur in response to information from research and technology development activities coupled with findings from precursor robotic missions.
- PP requirements for Mars missions will likely be very different than those used during Apollo missions. Early and regular coordination between the PP, scientific, planning, engineering, operations and medical communities is needed to develop practicable and effective designs for human operations on Mars. Coordination will bring numerous mutual advantages to the various programs such as identifying common needs for new technologies (e.g., among planetary science exploration, human mission operations, and PP).
- Significant amounts of materials will originate from human life support and mobility systems that can be

classified as forward contamination in both PP and scientific terms. All materials from the Martian environment are considered to be potential sources of back contamination (e.g., soil, airborne particulates). Forward and back contamination pathways include: leakage from habitat, airlocks and other vessels; egress/ingress of humans, materials and equipment; EVA operations; surface storage/disposal of wastes; gas venting (nominal and contingency); and thermal systems. Unintentional discharges may occur via events such as equipment failures, micrometeorite impacts, and rapid depressurization events.

Additionally, there was general consensus among participants regarding the need to establish requirements for both PP and scientific investigations early in the development cycle, as they significantly affect system design, technology trade options, development costs and possibly mission architecture. Of particular concern were the areas of discharge and disposal limits, backward contamination limits, and *in situ* resource utilization (ISRU). It is necessary to identify and define what will be regarded as contaminants by both PP and science communities. In addition, there is a clear need to develop a classification system of zones of biological, scientific, contamination and operational importance prior to and during human missions. Finally, data on protocols and systems used for quarantine of crew and hardware upon Earth return were identified as significant system drivers.

It was concluded that it was not possible to provide quantitative PP guidelines at the time of the workshop, as PP requirements will evolve in response to numerous factors. Instead, a tentative conceptual approach consistent with current PP requirements was proposed which asserts that human missions to Mars shall not affect or otherwise contaminate "special regions" of Mars, primarily through the use of cleaning operations and prudent landing site selection. It was also proposed that calculations based on this approach will determine the tolerable levels of contamination allowed for specific aspects of any particular human mission. Specific details of the approach are to be determined, but will involve close collaboration with the scientific community, and the evaluation of unavoidable levels of human-associated contaminants and their implications.

To facilitate the process of developing a quantitative set of PP requirements, the life support community indicated the need to further define initial material inventory, process products and by-products, release mechanisms associated with forward contamination, and the need to incorporate back contamination controls into system design and operations. The EVA community focused on the need to identify and control forward/backward contamination regarding suits and rovers from vent/leakage constituents. The environmental monitoring and control group noted the need for detection standards, response time requirements, and the challenges of identifying organisms that represent back contamination.

It was noted that long-duration lunar missions can provide a relevant test-bed for many mission technologies. It was suggested that mission planners address PP technology on the Moon in a manner that mimics Martian exploration, despite the comparatively relaxed PP requirements of lunar missions. Finally, it was cautioned that in planning long-term design and operations strategies, it will be critical to avoid pursuing two separate and costly technology pathways—one for the Moon and the other for Mars.

The intent of this presentation is to provide the participants of this workshop a brief summary of previous work on the topic[1,2], and to provide a base for furthering the overall investigation and PP requirements development. This presentation will provide an overall summary of the previous workshop that includes workshop objectives, starting assumptions, findings and recommendations. Specific result topics include the identification of knowledge and technology gaps, research and technology development needs, potential forward/backward contaminants and pathways, mitigation alternatives, and PP requirements definition needs.

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PLANETARY PROTECTION FOR HUMAN EXPLORATION MISSIONS: A FLIGHT SURGEON'S PERSPECTIVE. Jennifer Law, M.D., M.P.H.[1]
[1]NASA Johnson Space Center, Houston, TX.

Planetary protection will be a challenge for future human exploration missions beyond the Earth-Moon system. Historically, human spaceflight programs have not dealt with planetary protection issues since Apollo 14, the last manned mission to participate in the Lunar Quarantine Program after analysis of lunar materials demonstrated no biological threat. Whereas robotic missions are governed by NPR 8020.12D [1], which specifically defines the planetary protection categories and corresponding requirements and constraints that must be met, there is no analogous guidance for human missions. A significant experience gap exists in the implementation of planetary protection requirements between robotic and human missions.

Yet human missions will be orders of magnitude more complex than robotic missions. While the intent of planetary protection must still be met, the approach to preventing forward and backward contamination will have to be tailored to human missions. By their nature, human missions "will carry microbial populations that will vary in both kind and quantity," and "it will not be practicable to specify all aspects of an allowable microbial population or potential contaminants at launch" [2]. Human missions must emphasize protective measures to prevent contamination, rather than bioburden accounting and microbial reduction as in the case for robotic missions. Human factors must be taken into account. Waste from human metabolism, crew activities, and payloads must be contained.

At the same time, planetary protection needs to be taken into context with all the challenges facing human exploration missions. Vehicle considerations aside, there remains many poorly understood risks to the human body on such missions. NASA will fly its first year-long mission in 2015 in an effort to better understand human physiological adaptation to microgravity, although this longest mission to date will still be much shorter than a mission to Mars. The effects of hypogravity (e.g., 3/8 G on Mars) have yet to be characterized. Medical care for the crew will have to employ a different paradigm and greater crew autonomy. Crew health and wellbeing will be the utmost priority.

Opportunities exist for collaboration between the space medical and planetary protection communities. Both seek to understand the microbial composition of human missions and biochemical nature of planetary materials. Both have a vested interest in effective and efficient operations and protocols for contamination prevention, sample handling, and quarantine. Together, Space Medicine and Planetary Protection can develop guidelines and communicate them to the stakeholders. A collaborative approach will benefit both communities to support future human exploration missions.

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CAPABILITIES FOR PLANETARY PROTECTION: SAFEGUARDING THE CREW AND ENGINEERING SYSTEMS FOR HUMAN MISSIONS TO MARS. K. Venkateswaran, Jet Propulsion Laboratory, California Institute of Technology, 4800 Oak Grove Drive, Pasadena, CA; kjvenkat@jpl.nasa.gov

Planetary protection policies derive from international treaties whose goal is "to preserve our ability to study other worlds as they exist in their natural states; to avoid contamination that would obscure our ability to find life elsewhere—if it exists; and to ensure that we take prudent precautions to protect Earth's biosphere in case it does." Mandates are in place to minimize the likelihood of catastrophic outcomes as a result of human-associated cross-contamination between solar system bodies.

To meet planetary protection obligations, NASA needs:

- Integrated system technologies to protect human life from extraterrestrial microorganisms (should they exist) and to shield engineering systems from bio-corrosion.
- Assurance of compliance with evolving standards for planetary protection (both forward and backward contamination) relating to the human exploration of Mars.
- A sound technical basis to determine whether the inadvertent shedding of bio-contaminants from human explorers can be minimized to such a degree that the search for life could continue in an unobstructed, meaningful manner.

This presentation identifies a body of work to address NASA needs relative to microbial monitoring and controlling the harmful impact of microbial corrosion. One of the present knowledge gaps revolves around developing an integrated microbial monitoring system that is validated in a terrestrial Mars analog environment and ready for deployment on a human mission to Mars. Such a system needs to be developed and is essential for human missions to comply with requirements to avoid harmful contamination and thereby facilitate the search for extraterrestrial life. The integrated microbial monitoring system will bolster confidence in, and lend support to, planetary protection efforts, hardware reliability, and sustained crew health. By forewarning human explorers of any significant fluctuations in microbial burden, the system allows the crew to take immediate action to significantly diminish any threat to crew health, or deterioration of the habitation module resulting from bio-corrosion. This approach will strive to directly integrate the technologies proposed herein with those being developed for robotic Mars sample return missions, thereby providing a cradle-to-grave planetary protection implementation capability for human exploration.

Microbial biocontamination control in manned space habitats

Leys N.[1], Van Houdt R.[1]

[1] Belgian Nuclear Research Center SCK•CEN, Mol, Belgium

Natalie.Leys@sckcen.be

Past and current space missions in Earth orbit have demonstrated that men can survive and work in space for relative short durations. However, indoor microbial contamination in closed manned habitats leads to several environmental and health concerns, especially for longer duration space missions. Bio-contamination in confined spacecraft is a hazard and potential risk for the health of the crew and for the on-board equipment. Therefore, space agencies have implemented specific measures to prevent bio-contamination, to monitor it and to counteract it.

In this presentation, I will give an overview of our knowledge on the current procedures implemented by different space agencies to control bio-contamination in manned spacecraft, the scientific knowledge and technological gaps that we identified and our research efforts to support and possibly improve the current operational procedures. This presentation includes the work performed and planned in the international studies MISSEX, COMICS, THESEUS, BIOSMHARS and BIOSIS.

Prevention of bio-contamination should include rational habitat designs. Specific for the spread of biological aerosols, the development of a reliable model describing the bio-aerosol contamination spreading and development in closed manned habitat is important to pinpoint critical locations in a certain habitat design. BIOSMHARS is the first joint EU-Russia research project that addressed this issue. The first phase of the project aimed at developing, calibrating and validating a mathematical model to predict the dispersion of microbial bio-aerosols in the BIOS-facility, a closed environment in size and concept relevant for space, so far without human activity and under Earth conditions. The long-term objective of the BIOSMHARS-team is to develop a versatile and robust modelling tool for predicting airborne microbial contaminant dispersion and deposition in a manned spacecraft in flight.

Nevertheless, microbes are and will always be present in manned space habitats, as man is their most important source. But if we can better understand the survival and proliferation strategies of the indoor microbial communities in confined manned habitats, it will help us to manage and control their sources, dispersion and concentrations, in benefit of the space crew health and well-being. An overview of microbial communities found inside the International Space Station and the Concordia Antarctic base (an Earth analogue of a confined manned habitat) will be given. A detailed phenotypic and genetic analysis of bacterial water contaminants has taught us, how such bacteria of the *Cupriavidus* genus, can adapt to and manage to survive the silver sanitation procedures in ISS.

Within the THESEUS project an integrated life sciences research roadmap was written by Europe's scientific and industrial communities, in synergy with ESA, to (i) identify disciplinary research priorities, (ii) identify fields with high terrestrial application potential, and (iii) build a European network as the core of this strategy, for enabling future long-duration European human space exploration. This roadmap includes also recommendation on how to progress towards better microbiological quality control for the indoor environment in space in the future, and will be presented here.

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NASA'S INTERNATIONAL SPACE STATION: A TESTBED FOR PLANETARY PROTECTION PROTOCOL DEVELOPMENT. Bell, M.S.[1], Rucker, M.[2], Love, S.[2], Johnson, J.[2], Chambliss, J.[2], Pierson, D.[2], Ott, M.[2], Mary, N.[3], Glass, B.[4], Lupisella, M.[5], Scheuger, A.[6], Race, M.[7], [1]Jacobs, NASA Johnson Space Center, Mail Code XI3, Houston, TX 77058, USA, mary.s.bell@nasa.gov, [2]NASA Johnson Space Center, [3]Booz Allen & Hamilton, NASA Johnson Space Center, [4] NASA Ames Research Center, [5]NASA Goddard Space Flight Center, [6]University of Florida, [7]SETI Institute.

Introduction: Wherever humans go, they inevitably carry along the critters that live in and on them. Conventional wisdom has long held that it is unlikely those critters could survive the space environment, but in 2007 some microscopic aquatic animals called Tardigrades survived exposure to space [1] and in 2008 Cyanobacteria lived for 548 days outside the ISS [2]. Unlike the Mars rovers that were cleaned once and sent on their way, crew members will provide a constantly regenerating contaminant source. Are we prepared to certify that we can meet forward contamination protocols as we search for life at new destinations? What about the organisms we might reasonably expect a crewed spacecraft to leak or vent? Do we even know what they are? How long might our tiny hitch-hikers survive in close proximity to a warm spacecraft that periodically leaks/vents water or oxygen and how might they mutate with long-duration exposure [3, 4]? How will these contaminants migrate from their source in conditions encountered in space or on other planetary surfaces? This project aims to answer some of these questions by bringing together key stakeholder communities to develop a human forward contamination test, analysis, and integration plan. A system engineering approach to identify the experiments, analysis, and modeling needed to develop the contamination control protocols required will be used as a roadmap to integrate the many different parts of this problem – from launch to landing, living, and working on another planetary surface (Fig. 1).

Implementation: The focus of this road-mapping effort will be "what can we do now with what we have?" For example, the micro-organisms *inside* the International Space Station (ISS) are well-characterized but no one has ever swabbed an ISS external vent to find out what (if anything) has managed to get *outside*. We can swab ISS vents now, without having to wait for program direction or an Orion or a new rocket. If we take a sample and find nothing, that's good news! It means that our environmental control and life support (ECLS) vent filters may already meet forward contamination requirements. If we do find organisms *outside* the ISS, it will be interesting to see how they compare with what we typically find *inside*. Are they the same? Or have they mutated? What corrective measures can we take to prevent external contamination? Once we know what manages to escape a typical spaceship, we can expose it to various destination environments and see how it's likely to behave. Then we can go one step further, and test those organisms in a spacecraft-induced environment to understand whether proximity to a warm, venting spaceship makes a difference. That will tell us how far away we must land from a sensitive area to mitigate forward contamination. We could also bring the modeling community into play and overlay destination weather models onto bacterial growth models to estimate how far microbes could be transported by, say, a small dust storm on Mars. Another opportunity might be to take a sample from an Exploration Extra-Vehicular Activity (EVA) Suit during development testing and follow similar steps as outlined above: what organisms come out of a suit vent or leak from the suit? How close can EVA crew be to a sensitive site without compromising the science objectives? Data would tell us what modifications might be required to the suit *now*, early in the development phase, and avoid an expensive redesign later.

Technical Objectives and Outcomes: This project has four technical objectives:

1. Develop a detailed test plan to leverage existing equipment (i.e. ISS) to characterize the kinds of organisms we can reasonably expect pressurized, crewed volumes to vent or leak overboard;
2. Develop an analysis plan to study those organisms in relevant destination environments, including spacecraft-induced conditions;
3. Develop a modeling plan to model organism transport mechanisms in relevant destination environments;
4. Develop a plan to disseminate findings and integrate recommendations into exploration requirements & operations (Ops).

Technology Readiness Level (TRL): There will be different TRLs for different aspects of this project but because the emphasis is on utilizing what we currently have, TRLs are expected to be fairly high (ISS utilization, for example).

Alignment to NASA and Johnson Space Center Strategic Objectives: This work will influence explo-

ration-class life support and EVA suit design (including whether closed loop is required to meet planetary protection), and aid in developing crew health protection strategies. This work also supports one of NASA's Strategic Knowledge Gaps: Microbial survival, Mars conditions [5].This project supports JSC Strategic Plan Goal #1: Lead Human Exploration, specifically Strategy 1.3 (Extend human exploration beyond LEO) by providing a roadmap to characterize human forward contamination. With that piece of the puzzle in place, we can better understand hardware and operational implications at various destinations beyond LEO. This work also aligns to NASA's strategic goals for exploration as designated in *NASA's Space Technologies Roadmaps and Priorities* [6] specifically Technology Area Breakdown numbers 6.0: *Human Health, Life Support and Habitation Systems,* and 7.0: *Human Exploration Destination Systems Roadmap* [7].

Project Infusion Path: This project's primary deliverable is a JSC-numbered document that will serve as the roadmap for many spin-off efforts, such as ISS utilization tests, advanced EVA and ECLSS development, and crewed operational procedures. This work will link JSC's hardware developers with scientific communities across the Agency, and will provide information and guidance to commercial hardware developers planning crewed exploration missions.

Fig.1. This project will provide a roadmap to integrate planetary protection requirements into the design of engineering systems necessary for human exploration of a variety of destinations.

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PHOBOS AND DEIMOS: PLANETARY PROTECTION KNOWLEDGE GAPS FOR HUMAN MISSIONS.

Pascal Lee[1,2,3] and Kira Lorber[1,4], [1]Mars Institute, NASA Ames Research Center, MS 245-3, Moffett Field, CA 94035-1000, USA, pascal.lee@marsinstitute.net, [2]SETI Institute, [3]NASA ARC, [4]University of Cincinnati..

Summary: Phobos and Deimos, Mars' two moons, are associated with significant planetary protection knowledge gaps for human missions, that may be filled by a low cost robotic reconnaissance mission focused on elucidating their origin and volatile content.

Introduction: Phobos and Deimos are currently considered to be potentially worthwhile destinations for early human missions to Mars orbit, and possibly in the context of longer term human Mars exploration strategies as well [1] (Fig. 1). Until recently, it was widely considered that planetary protection (PP) concerns associated with the exploration of Phobos and Deimos would be fundamentally no different from those associated with the exploration of primitive NEAs [2], as the preponderance of scientific evidence suggested that 1) there was never liquid water on Phobos and Deimos, except possibly very early in their history; 2) there is no metabolically useful energy source except near their heavily irradiated surface; 3) there was likely never sufficient organic matter on or in these bodies to support life within the zone where metabolically useful energy is available, because of space radiation; 4) subsequent to the disappearance of liquid water, these bodies have not been subjected to extreme temperatures (i.e., > 160°C), except near the irradiated surface zone as a result of impacts; 5) there is, and was, sufficient irradiation for biological sterilization of terrestrial life form near the surface; and 6) there has likely been a natural influx to Earth, via meteorites, of material equivalent to a sample returned from the target body.

However, two recent developments relating to the origin of Phobos and Deimos are prompting a revisit of PP assessments for these bodies:

Giant Impact Hypothesis. In addition to the two classical hypotheses concerning the origin of Phobos and Deimos (namely that they might be captured small bodies from the outer main belt, or remnants of Mars' formation), a third hypothesis is now commonly cited: that they might be reaccreted Mars Impact ejecta. The latter includes the possibility that Phobos and/or Deimos formed less than 0.5 Ga ago, and are made of Martian crustal material ejected from Mars's near-surface environment by a large impact. Given the possibility of this scenario (if not its plausibility: giant impacts are unlikely in recent times), it can no longer be said with the same confidence that there is a preponderance of evidence for the six inferences listed above.

Figure 1: Deimos (left) and Phobos (right), to scale. Deimos is 15 km long, and Phobos, 27 km long. Both moons have, to first order, a D-type spectrum: they are very dark (albedo ~ 0.07) and very red. (NASA MRO).

Extinct Comet Hypothesis. As a variant of the "captured small body from the outer main belt" hypothesis, it has long been suggested that Phobos and/or Deimos might be captured comet nuclei. (now inactive or extinct). Consistent with this idea, some grooves on Phobos (those resembling crater chains, or *catenas*) are interpreted as fissures lined with vents through which volatiles were once outgassed [3] (Fig. 2).

Figure 2: Crater chain-like grooves on Phobos have been interpreted as fissures with vents through which volatiles once outgassed. (NASA Viking Orbiter 1).

While the grooves on Phobos could have an entirely different origin, recent outgassing activity detected around D-type NEA 3552 Don Quixote revives the extinct comet hypothesis for Phobos and Deimos. The key facts and observations are as follows:

a) *All 3 are D-type objects*: Phobos, Deimos, and NEA 3552 Don Quixote all present, to first order, a D-type spectrum, ie., they are very dark and very red in the visible and near-IR;

b) *D-types are rare in the inner solar system:* Only 36 NEAs are known to be of D-type, i.e. only 1.5% of NEAs whose spectral type has been determined;

c) *Phobos & Deimos are exceptionally large as inner solar system small bodies:* If Phobos and Deimos were to be included in the NEO population, they would rank #3 and #5 in size, respectively;

d) *NEA 3552 Don Quixote is exceptionally large as well.* Don Quixote would rank #4 in size, i.e., it is intermediate in size between Phobos and Deimos (Fig. 3). Thus, although D-type objects are rare among NEAs, three of the five largest small bodies in the inner solar system have a D-type spectrum;

Figure 3: Deimos (top left), NEA 3552 Don Quixote (bottom left), NEA Itokawa (top right), and Phobos (bottom right), to scale. (NASA & JAXA).

e) *NEA 3552 Don Quixote is a comet nucleus.* Ever since Don Quixote's discovery, it has been suggested that it might be an extinct comet, as it revolves around the sun on a highly inclined (30°), Jupiter and Mars-crossing orbit, and is spectrally akin to small bodies found in the outer main belt and beyond. In early 2014, however, 3552 Don Quixote was observed to display comet-like activity, with a coma and tail associated with CO_2 emission [4]. Rather than being extinct, it is a moderately active comet nucleus.

Thus, given that Phobos and Deimos are similar to 3552 Don Quixote in both size *and* spectral type (albedo *and* color), and these have uncommon values among inner solar system small bodies (they are large objects and of spectral type D), the hypothesis that Phobos and Deimos might be captured comet nuclei that are now extinct or largely inactive merits serious consideration. Their low bulk densities, which is generally attributed to an interior with high macroscopic porosity, is also consistent with a volatile-rich interior. If a volatile-rich interior is a likely possibility, then PP assessments of Phobos and Deimos would need be to revised.

While surface and near-surface materials on Phobos and Deimos are likely to be depleted in volatiles as a result of diffusive exposure to space and impact processing, the deeper interior might remain volatile rich. As new technologies (such as plasma drilling [5] (Fig. 4)) designed to access and sample the deeper interior of planetary bodies, including airless small bodies, are now emerging, human exploration will likely not remain confined to sampling surface and near-surface materials, but will likely include interacting with deeper interior materials on Phobos and Deimos, including potential volatiles.

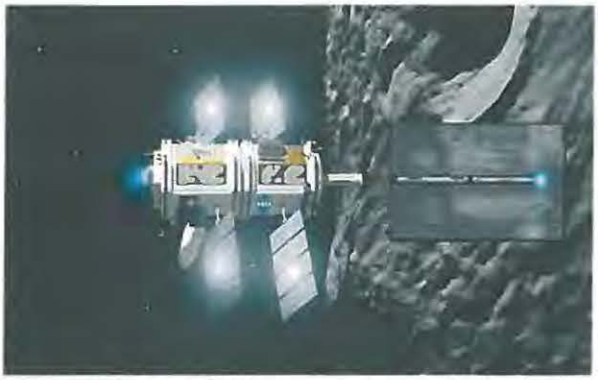

Figure 4: Plasma drilling opens the possibility for human explorers to access and sample, the deeper interior of small bodies such as Phobos or Deimos (Zaptec).

Conclusion: The volatile content, and therefore the origin and evolution, of Phobos and Deimos, are major PP knowledge gaps for future human missions to these bodies. To better constrain PP requirements associated with their exploration, a robotic reconnaissance mission to Phobos *and* Deimos focused on investigating their origin and assessing their volatile content is recommended. Short-term options include low-cost and low-risk Discovery class missions such as PADME (Phobos And Deimos & Mars Environment) [6].

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OVERVIEW OF MICROBIAL MONITORING TECHNOLOGIES CONSIDERED FOR USE INSIDE LONG DURATION SPACEFLIGHTS AND PLANETARY HABITATS. M. C. Roman[1] and C. M. Ott[2], [1]NASA Marshall Space Flight Center, Mail Code FP10, Huntsville, Al 35812 monsi.roman@nasa.gov; [2] NASA Johnson Space Center, 2101 Nasa Parkway, Houston, TX 77058 c.m.ott@nasa.gov

Abstract: Humans have been exploring space for more than 40 years. For all those years, microorganisms have accompanied both un-manned spacecraft/cargo and manned vessels. Microorganisms are everywhere on Earth, could easily adapt to new environments, and/or can rapidly mutate to survive in very harsh conditions. Their presence in spacecraft and cargo have caused a few inconveniences over the years of human spaceflight, ranging from health problems, life support systems challenges, and material degradation. The sterilization of spacecraft that will host humans in long duration mission to prevent forward contamination would be a costly operation that will not provide a long-term solution to the unavoidable microbial colonization of the vessels. As soon as a human is exposed to the disinfected spacecraft, microorganisms will start populating the new environment. As the human presence in space increases in length, the risk from the microbial load to hardware and crew will also increase. Mitigation of this risk of forward contamination involves several different strategies that will include minimizing the microbial load (in numbers and diversity), understanding where microorganisms can be transferred from the crew/vehicle to the planet, and microbiological monitoring to verify our successful mitigation. NASA has been looking at microbial monitoring technologies that could be used in long duration missions. This presentation will provide an overview of the microorganisms found on spacecraft and microbial monitoring technologies that are been considered for use inside spacecrafts and planetary habitats.

Current Trends of High-Throughput Methods for Planetary Protection Requirements Associated with a Human Mission. F. Karouia[1,2], K. Peyvan[3], O. Santos[1], and A. Pohorille[1,2]. [1]NASA Ames Research Center MS 239-4 Moffett Field CA 94035 (fathi.karouia@nasa.gov); [2]University of California San Francisco; and [3]Peyvan Systems.

Introduction: Future human extraterrestrial missions, and missions to Mars in particular, will be extremely challenging to operate. Such mission will require a sophisticated bioregenerative life support system while in orbit and efficient *in-situ* utilization systems while on the surface to manage and produce all the needed resources (oxygen, water, waste, etc.) to support human activities [1-2]. Most likely consortia of specialized, adapted, or engineered microorganisms will be intentionally introduced or used for such efforts [3]. Furthermore, the presence of humans will have a considerable impact on the level of bioburden, as it has been established that a human body carries 100 times more microbes than human cells [4].

Therefore, in order to fulfill planetary protection requirements regarding forward and backward contamination, microbial population in both kind and quantity will have to be monitored and evaluated throughout the mission. The emergence of advanced molecular assays and high-throughput techniques of system biology is of special importance to planetary protection. Using such systems it can efficiently assessed what are the levels of bioburden, qualitatively or quantitatively estimate the level of biodiversity, and track genetic changes induced by the harshness of space environments [5]. For example, cytological (flow cytometry) and Biomarker (Limulus Amebocyte Lysate and ATP assays) methods have been used to assess the level of bioburden. However, high-throughput mass spectrometry and PCR-based techniques either individually or in conjunction with DNA microarrays are more suitable approaches to infer diversity. Finally, sequencing methods are capable of identifying genetic changes, induced by space radiation in particular, that occur in microbial communities during their journey [6-8].

The effects of space environments on biological systems are influenced by many factors and should be studied using global, integrative methods. This, in turn, implies that "omics" approaches are not only helpful but are indispensable for planetary protection [5]. We will discuss which "omics" technologies are currently amenable to adaptations for space applications and how these adaptations can be achieved [8]. We will review ongoing efforts aimed in this direction and discuss scientific benefits that they might bring. In particular, we will argue that, with sufficient commitment, at least some instruments for high-throughput methods could be ready for deployment on-board spacecraft in the next few years. Once developed and deployed, "omics" tools can be used for a wide variety of high-value studies on biological systems ranging from microorganisms to humans for planetary protection and human health purposes.

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NEAR REAL-TIME QUANTITATION OF VIABLE MICROORGANISMS FOR PLANETARY PROTECTION AND CREW HEALTH. N.R. Wainwright, Charles River Laboratories, 1023 Wappoo Rd., Suite 43-B, Charleston, SC 29407, norm.wainwright@crl.com

Introduction: The need for rapid assessment of microbial bioburden is practically universal. This is as true for Planetary Protection and Crew Health as it is in the Biopharmaceutical and Food Sciences. For Planetary Protection, the knowledge of when minimum acceptable levels of contamination are exceeded is critical to keeping flight hardware in specification as manufacturing and assembly proceed. It is equally critical to mission science, especially when potential life detection science that may be impacted. For Crew Health, presence of potential pathogens are of primary concern. Whether detection methods are focused on preventative measures to keep environments clean, or on clinical diagnostic procedures that could help diagnose and treat infection, procedures and equipment to rapidly and simply provide information to the crew is paramount. We have developed a technology for the Biopharmaceutical field to detect and count viable microorganisms in a sample, without the need to culture, in about an hour. Sensitivity is as low as a single cell in a given volume. The robustness and portability of the system is amenable to both Planetary Protection and Crew Health requirements.

Background: Earlier work focusing on non-culture methods included measurement of ATP by the luciferin/luciferase system and bacterial endotoxin (LPS or lipopolysaccharide) by Limulus Amebocyte Lysate (LAL). Both of these methods have been approved as ancillary Planetary Protection testing methods [1]. From 2006 – 2009, we tested LAL on the International Space Station (ISS) as a technology demonstration (LOCAD-PTS). Much useful information and valuable experience was gained during that study in quantifying microbial contamination on surfaces [2,3,4]. However, we always realized that direct, selective measurement of <u>viable</u> organisms by non-culture methods was the ultimate objective.

New Technological Approach: To achieve the sensitivity required in the minimal time available, we designed a system with three major components, sample acquisition, fluorescent viability staining and laser scanning.

Figure 1. Full system shown with door open, revealing the rotary stage and optics module. A touch screen on top controls the unit. Approximate size: 1 cubic foot.

Sample acquisition. Samples should be suspended in an aqueous solution, either directly from a liquid or extracted from a surface or solid material. These can be loaded directly in a 10 µl capillary, or concentrated by deposition on a filter membrane, shown below.

Figure 2. Left: 100 ml filter cup assembly; center: membrane with cup removed; right: 10µl capillary.

Fluorescent viability stain. Selective visualization of viable cells relies on a combination of dyes that accumulate in cells and a quencher that is only permeable to dead cells. A number of dye / quencher combinations are available that are directed to a number of targets, such as nucleic acids, redox reactions and esterase. In addition, fluorescent particles (0.8 µ) can be included as positive controls. Data from several examples will be presented.

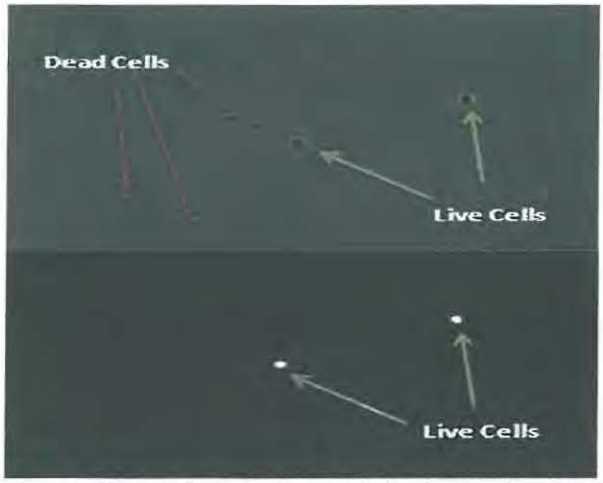

Figure 3. Upper panel: live and dead cells in phase contrast microscopy; lower panel: same field under fluorescence only.

Laser scanner. The scanner has a unique rotary mechanisms capable of scanning both membrane filters and capillaries. The system is comprised of a rotary stage on which the medium is held, a laser directing a collimated beam onto the medium to excite the fluorochrome stained cells, and an optic module housing three photomultipliers, each capturing a portion of the total emitted light spectrum.

Figure 4. The rotary stage is shown with three membranes being scanned simultaneously.

Following a scan, software analyzes the number, position, color and intensity of fluorescence for each cell or control particle. Very strict criteria are set to eliminate false positives. Scan time is approximately 20 minutes for the three membranes (or four capillaries). Coupled to 15 – 30 minute stain time, the total assay time is less than an hour. As a validation of the process, the membrane filters may be placed on nutrient agar to grow and count colonies. A number of examples will be presented.

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COMPREHENSIVE AND SENSITIVE MICROBIAL DETECTION USING A BROAD SPECTRUM DETECTION MICROARRAY. C. Jaing, S. Gardner, K. McLoughlin, J. Allen, J. Thissen, N. Be, T. Slezak, Lawrence Livermore National Laboratory (7000 East Ave., Livermore, CA, 94550, jaing2@llnl.gov).

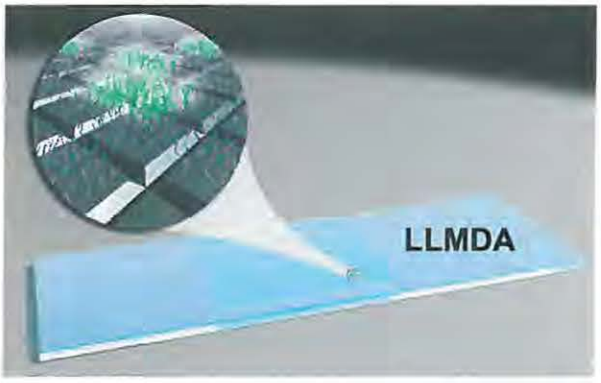
Molecular detection of microbes: Recent advances in genomic-based technologies have revolutionized the field of microbial ecology and their influence on infectious diseases in human [1]. PCR and DNA sequencing approaches have been widely used for pathogen detection and characterization. PCR assays are limited, in that only a single or few organisms can be investigated per assay, with potentially high false-positive rates. While DNA sequencing can identify a larger scope of organisms, current DNA sequencing analysis methods are lengthy, costly and require significant computational time, and there is a lack of bioinformatic tools to rapidly identify and quantify abundances of species identified in a sample. In an effort to improve high-throughput analysis and detection, we have developed an innovative microarray platform called the Lawrence Livermore Microbial Detection Array (LLMDA) to probe for all known microbiological agents for which whole genomes, segments and plasmid sequences are available [2].

The LLMDA: The LLMDA technology can be applied to large numbers of environmental and clinical samples in a highly sensitive, specific, and cost-effective fashion. The recently updated LLMDA contains 180,000 probes designed to detect genomic DNA and cDNA from all currently sequenced microbial pathogens, a total of 10261 species, including 4219 viral, 5367 bacterial, 293 archaeal, 265 fungal, and 117 protozoan species that were sequenced through June, 2013. This microarray targets both conserved and unique genomic regions of sequenced microbial strains. It has higher probe density and broader taxonomic representation of viral, bacterial, and fungal genome sequences than other published array designs [3]. The automated data analysis algorithm, Composite Likelihood Maximization (CLiMax), is integrated with a web interface that enables LLMDA data analysis within 30 minutes.

LLMDA in human health applications: LLMDA was recently established as a potential diagnostic platform for identification of viral pathogens in human clinical samples [4]. As little as 5 input genome copies (≥1,000 copies/ml) of BK polyomavirus were clearly detected by LLMDA in urine samples after phi29 amplification [4]. Additionally, the LLMDA successfully identified other viral agents such as human papillomavirus, human herpesviruses, enteroviruses, and adenoviruses in a variety of human sample types including nasal swabs, urine, stool, serum, and cerebrospinal fluid [4]. In an another study, LLMDA identified human herpes virus 8, or Kaposi's sarcoma-associated virus from human bladder cancer samples, indicating viral infection may play a role in tumorigensis of bladder cancer [5]. In a collaborative study with the Naval Medical Research Center, the LLMDA was used to profile the microbial contents from wounded soliders. The array detected at least one pathogen from 30% of the samples that were negative by microbiological culture testing [6]. Additionally, the LLMDA has found that certain bacteria, such as *Acenitobacter baumaii* are assoiated with wounds that failed to heal, wherees *E.coli* species are associated with wounds that did heal, indicating that microbial profiles could be linked to wound outcome and could be used to better inform treatment decisions.

LLMDA in detecting vaccine contamination and drug safety: In 2010, the LLMDA identified a porcine circovirus in the Rotarix vaccine [7,8]. This was the first study to report an adventitious contaminating virus from childhood vaccines, which demonstrated that LLMDA is a sensitive and powerful technology for vaccine safety monitoring. Currently, the LLMDA is being evaluated as a tool for risk assessment in pharmaceutical industry.

LLMDA in environmental monitoring: The LLMDA technology has been evaluated in microbial detection in environmental air and soil samples. LLMDA detected as little as 100 genome copies of *B. anthracis* in spiked environmental air and soil samples, similar in sensitivity to next-generation metagenomic sequencing [9]. In a collaborative study with JPL, the utility of LLMDA in detecting bacteria from environmental samples collected at the ISS was evaluated. The LLMDA successfully detected *P. acnes*, *S. aureas*, *S. epidermidis* and *S. cohnii*, confirming previous results by sequencing.



Comparison to DNA sequencing: The advantage of the LLMDA in speed and cost over next-generation sequencing was recently demonstrated in the field of archaeology [10]. The array successfully identified previously verified bacterial human pathogens, including *Vibrio cholerae* (cholera) in a 19th century intestinal specimen and *Yersinia pestis* ("Black Death" plague) in a medieval tooth, which represented only minute fractions (0.03% and 0.08% alignable high-throughput shotgun sequencing reads) of their respective DNA content. LLMDA can identify primary and/or co-infecting bacterial pathogens in highly degraded and complex ancient samples, thereby serving as a rapid and inexpensive screening tool to study health across both space and time. The LLMDA is currently being developed into a 96-well format microarry to facilitate even higher throughput environmal and clinical microbiome studies.

The Livermore Metagenomics Analysis Toolkit: Livermore Metagenomic Analysis Toolkit (LMAT) is a collection of software tools designed to identify the genes and organisms present in a shotgun metagenomic sample [11]. LMAT maintains the most complete collection of microbial genomes and genes publicly available in a rapidly searchable form. The database includes all publically sequenced strains of viruses, bacteria, archaea, fungi, protozoa, and eukaryote mitochondria and an extensive collection of human genetic variation derived from the 1000 human genomes project. The database covers 12,632 species and stores 116 gigabases of searchable genomic data, which is roughly 3 times larger than any other published searchable database available for metagenomic search. LMAT ensures consistently fast runtimes and maintains high accuracy by using strain-specific k-mers (where k is typically 20 nucleotides), searching for common elelments only once. LMAT has been used to analyze many terabases of metagenomic data and provide novel insights on organisms present that were not previously considered. For example, LMAT was recently run on the complete 18 terabase collection of human microbiome shotgun metagenomic samples and discovered a large collection of previously unidentified human genomic contaminants as well as candidate novel microbes.

Summary: We have developed a suite of genomic and bioinformatic tools including the Lawrence Livermore Microbial Detection Array and Metagenomics Analysis toolkit, scalable and easily adaptable to support space biology and the human extraterrittrial missions. The tools are highly sensitive and specific for genomic analysis and molecular characterization of human health and microbiomes, and to profile microbial contents in the environment.

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Current Progresses Of Midass: The European Project For An Automated Microbial Identification Instrument

Claude Mabilat[1*], Hafid Abaibou[1], Robert Lindner, Stephanie Reffestin[3] and Christophe Lasseur[3]
[1]bioMérieux S.A. (France), [2]NTE-Sener (Spain), [3]ESA-ESTEC (The Netherlands)

*: Christophe.lasseur@esa.int

For the long term manned missions, microbial contamination is a major risk for exobiology research, crew members and hardware. Rapid molecular biology techniques offer an attractive alternative to traditional culture-based methods. They allow fast time to results for contamination detection and quick implementation of appropriate corrective action when required. However, to date, there are no such available system due to the technical challenges required to meet the sensitivity and specificity needs of the test and the requirement for full automation, from sampling to results interpretation. In response to this, over the last decade, the European Space Agency (ESA) and bioMérieux initiated a co-development of MIDASS, the world's first fully automated system for the monitoring of the environmental microbial load in confined spaces, including clean rooms and hospital wards. The system is based on molecular technologies (sample preparation/amplification/detection) and enables rapid and simple determination of the microbiological contamination level in less than 3 hours. It relies on NASBA-amplification for the detection of selected micro-organisms (indicators or pathogens) at determined risk-levels (200 and 1 CFU /m^3 air, respectively). Successful progresses were recently made for the space-application workpackage of this project: a lab-on-a-card design for air-testing in a first scope was endorsed by a successful ESA Preliminary Design Review, paving the way to spatialization steps (phases C and D). Data will be presented with regards to system design and biological performances.

The Sloan Foundation Microbiology of the Built Environment program: What's there? Where does it come from? And what does it mean? Paula J. Olsiewski, Alfred P. Sloan Foundation, 630 Fifth Avenue, Suite 2200, New York, NY 10111 (olsiewski@sloan.org)

Introduction: From 2000-2010, the Alfred P. Sloan Foundation funded a program that provided $45MM in grants to reduce the threat of bioterrorism. An important aspect of that program was funding work to make buildings safer against microbial and other biological threats. Through this work, it became apparent that there was no baseline or catalog for indoor microbes. As a result, in 2004, Sloan began supporting basic research in this area by coaxing prominent life scientists Norman Pace and J. Craig Venter to move from studying natural outdoor environments to indoor built environments.

The Microbiology of the Built Environment Program:

Since 2004, the Alfred P. Sloan Foundation has provided over 100 grants for a total of $38MM in funding. Sloan grantees have been asking three main questions: 1. What's there? 2. Where does it come from? 3. What does it mean? To address these questions, Sloan grantees have been using both life science and building science tools. They also have been developing new methodologies. It is expected that findings from this program will lead to better approaches for designing, constructing, and operating buildings. Grantees include engineer and ecologist Jessica Green of the University of Oregon, ecologist Jack Gilbert of the University of Chicago, and environmental engineer William Nazaroff of UC Berkeley. The presentation will share some of the recent findings and describe plans for future grant-making opportunities.

SURFACE SAMPLING AND DETECTION INVESTIGATIONS AT THE CDC. L. J. Rose[1] and A. D. Coulliette[2], ([1]Division of Healthcare Quality Promotion, Centers for Disease Control and Prevention, 1600 Clifton Rd, MS C-16, Atlanta GA 20329. LRose@cdc.gov) ([2]Division of Healthcare Quality Promotion, Centers for Disease Control and Prevention, 1600 Clifton Rd, MS C-16, Atlanta GA 20329. ACoulliette@cdc.gov).

Introduction: The Environmental and Applied Microbiology Team is a group of microbiologists within the Division of Healthcare Quality Promotion that is tasked with investigating disease outbreaks in healthcare settings. During the course of these investigations, questions have arisen as to the efficiency of the available sampling and detection methods. In order to better understand and optimize sampling and detection of microorganisms, the team has undertaken several applied research endeavors. This presentation will summarize and discuss some of our work and findings.

Sampling devices: The team has investigated the efficiency of devices such as swabs [1], sponges [2], gauze wipes and vacuum devices [3] to pick up a variety of organisms from several surface types and the efficiency of the devices to release the organisms into solution. Organisms evaluated include multi-drug resistant bacteria such as Methicillin resistant *Staphylococcus aureus* (MRSA), *Clostridium difficile* spores, and *Acinetobacter baumanii,* as well as biothreat organisms like *Bacillus anthracis* spores.

Surface: The efficiency of the sampling and detection is influenced by the characteristics of the surface materials the organisms are sampled from, such as roughness, porosity and hydrophobicity. Each sampling device also has a limit as to the surface area that is optimum to sample before efficiency is lost. We investigated the use of composite sampling for one study[4], in which each side of one sponge sampling device was used for several sites in a room.

Detection: Culture has been the primary method of detection for the organisms investigated, since we have found that the limit of detection is significantly lower than for detection with molecular methods such as qPCR, and because we need to know the organism is viable. The sampling and detection method should therefore be chosen based on the objectives of the sampling and how the results can inform decisions and actions required to protect public health.

Culture Gaps. Growth of the target organisms without overgrowth of background organisms has been a challenge. Though selective media is available for some of our target organisms, they are not always time efficient or effective for a given environmental consortia. In addition, after disinfection or dessication, organisms may be injured and viable but non-culturable, leading to false negative detection results.

Molecular Detection Gaps. Limit of detection is a significant gap. Though PCR assays can be sensitive, since typically a volume of only 5µL is in the reaction well, concentration of the sample eluate without concentration of background organisms and/or assay inhibitors is required, and has proven to be a challenge.

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ENVIRONMENTAL CONTROL AND LIFE SUPPORT SYSTEMS FOR MARS MISSIONS – ISSUES AND CONCERNS FOR PLANETARY PROTECTION. D.J. Barta and M.S. Anderson, NASA Johnson Space Center, 2101 NASA Parkway, Houston, TX, 77058. daniel.j.barta@nasa.gov, molly.s.anderson@nasa.gov

Introduction: Planetary protection represents an additional set of requirements that generally have not been considered by developers of technologies for Environmental Control and Life Support Systems (ECLSS). Planetary protection guidelines will affect the kind of operations, processes, and functions that can take place during future human planetary exploration missions.

Forward Contamination: Forward contamination concerns will affect release of gases and discharge of liquids and solids, including what may be left behind after planetary vehicles are abandoned upon return to Earth. A crew of four using a state of the art ECLSS could generate as much as 4.3 metric tons of gaseous, liquid and solid wastes and trash, and 2 metric tons of used hardware during a 500-day surface stay. This rate includes the fact that state-of-the-art ECLSS technology such as that currently on the International Space Station includes partial waste recycling. Certainly, further closure of ECLSS systems will be of benefit by greater reuse of consumable products and reduced generation of waste products. But how must these wastes be managed? It will be cost prohibitive to return these wastes to Earth. Process technologies to treat, sanitize, mineralize or permanently store these products will add to launch mass requirements.

It can be presumed that planetary protection will affect technology development by constraining how technologies can operate: limiting or prohibiting certain kinds of operations or processes (e.g. venting); necessitating that other kinds of operations be performed (e.g. sterilization; filtration of vent lines); prohibiting what can be brought on a mission (e.g. extremophiles); creating needs for new capabilities/technologies (e.g. containment).

Although any planned venting could include filtration to eliminate micro-organisms from inadvertently exiting the spacecraft, it may be impossible to eliminate or filter habitat structural leakage. Filtration will add pressure drops impacting size of lines and ducts, affect fan size and energy requirements, and add consumable mass. Contingency operations such as cabin depress for fire response may have to be reconsidered, necessitating additional hardware such as scrubbers for post-fire cleanup.

Technologies that may be employed to remove biomarkers and microbial contamination from liquid and solid wastes prior to storage or release may include mineralization technologies such as incineration, super critical wet oxidation and pyrolysis; however these come with significant penalties for mass, power and consumables. Additionally, operations of current and historical human spacecraft without planetary protection needs have not led to strong demand for these technologies, and their development lags behind other functions. More detailed knowledge is needed for what specific chemical and organic materials are acceptable to be vented or left behind without treatment. Are there concerns for non-biological contamination for reasons other than planetary protection, such as for protection of science?

Backward Contamination: Developers of life support systems have several concerns related to backward contamination, both physical and biological. The life support system may be an important step in minimizing the backward contamination, or have to react to that contamination happening.

The life support system is a critical part of minimizing the effects of backward contamination from dust or regolith on the Martian surface. Characterizing the properties of Martian dust before human missions is clearly important. Knowing the impacts on human health will set the limits of allowable contamination, and knowing other characteristics will help design efficient technologies for control and removal of the dust. However, it's also very important to estimate the amount of dust that will be brought into the habitat during nominal or contingency operations. Suitports and other layered defense strategies can minimize the dust or regolith brought deeper into the habitat, but it will not completely eliminate it. While it may be obvious, it's important to point out that the vehicle life support system removes dust or regolith after the crewmember has been exposed. It will not be a perfect barrier. Medical communities should be assuming some level of contact between the crewmembers and the Martian environment is inevitable.

A second backward contamination issue important to the design of the life support system is the use of in-situ resources. The essential issue is whether medical experts will allow human consumption (through drinking or atmospheric contact and metabolic use) of consumables generated from the Martian surface and atmosphere. Are there additional monitoring or measurement requirements that need to be placed on either the ECLSS or ISRU system to validate quality? There is a knowledge gap (at least within the ECLSS community) as to what contaminants will be present in ISRU generated consumables that would be different from either Earth supplied consumables or resources

from recycled wastes. The contaminants will likely vary depending on process (melting ice vs. chemically reacting atmospheric components). If particular types of consumables will never be acceptable, that places an important constraint on mission and system architecture. The standards currently in place for water and air quality are typically based on describing allowable quantities for expected contaminants. The requirements and specifications used in ISS are likely not sufficient to describe the requirements for fluids and environments during exploration missions. For example, perchlorates and chromium do not appear in the Spacecraft Water Exposure Guidelines (SWEGs).

Since some backward contamination is likely inevitable, the need for quarantine is an important consideration for life support design. If a crewmember develops symptoms of illness after exposure, will the life support system have to provide an isolated atmosphere and water system to act as a medical quarantine? This essentially results in a doubling of life support functions and significant increase in vehicle size. If the quarantine is short term, simpler units may be used, but duration becomes highly important. And if waste must be disposed of after the quarantine period as if it were a biohazard, that also introduces difficult new requirements. Quarantine may also be provided by separation through the operation of distinct mission vehicles, with an attempt to minimize cross contamination when the crew transfers from the surface elements, to an ascent vehicle, to a transit habitat, and back into the Orion vehicle. The timing of these moves is based on orbital mechanics, and cannot be adjusted to wait out an illness. Thus, medical quarantine may need to be considered for all vehicle elements.

Closing Comments: Ultimately, there will be an effect on mission costs, including the mission trade space when planetary protection requirements begin to drive vehicle deisgn in a concrete way. Planetary protection requirements need to be considered early in technology development and mission programs in order to estimate these impacts and push back on requirements or find efficient ways to perform necessary functions. It is expected that planetary protection will be a significant factor during technology selection and system architecture design for future missions.

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Human Life support by microbes in space

Leys N.[1], Janssen P.[1], Monsieurs P.[1], Mastroleo F.[1]
[1] Belgian Nuclear Research Center SCK•CEN, Mol, Belgium
Natalie.Leys@sckcen.be

To ensure that man can survive and work in space for long durations (e.g. on the moon or on Mars) and to reduce the costs of these expeditions to feasible levels, technological and scientific breakthroughs in life support systems and related recycling technologies are required. A number of recommendations how this could be developed in the future were recently formulated by us, together with a group of European experts, within the THESEUS roadmap.

The large potential of bio-based systems for human life support in space was included. On Earth, life exists thanks to microbes, and we use them every day for producing our food, for our health and well-being, and for maintaining a viable environment. Such water and soil microbes will also have useful applications in space, including the recycling of valuable mineral and organic substrates. In this presentation, we discuss how bacteria can be used in closed life support systems to support human life in space, taking the MELISSA system as model. Since 1989, the MELiSSA project groups a number of European and Canadian scientists and industrial partners to investigate and demonstrate how microbes can transform organic waste into fertilizers for algae and plant cultures, which in turn will produce oxygen, purify drinking water, and provide fresh food for man in space habitats. In parallel, we are looking, together with a team of geo-microbiology experts, into the potential application of microbes to make planetary regolith suitable for agriculture.

That microbes can survive and proliferate in space habitats is well documented. But for microbes to be successfully used in biotechnological applications in space, their interaction with water, soil, and rock substrates under space conditions has to be studied, particularly because these microbes will be exposed to extended periods of extreme conditions including high radiation, low gravity, low pressure, low and/or high temperatures, and complex chemical compositions. Thus, in this presentation, also the challenges to optimize the desired bacterial activity under space conditions will be discussed. We will give a small overview of what is known, from former flight and ground experiments, on the effects of such space environmental factors on the survival, proliferation, and metabolic activity of water- and soil microbes, or ecologically engineered communities thereof. Our plans for future flight experiments to further investigate this issue will also be explained.

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EXTRAVEHICULAR ACTIVITY AND PLANETARY PROTECTION. J. A. Buffington[1] and N. A. Mary[2], [1]EVA Exploration Architecture Lead, EVA Management Office, NASA Parkway/FX, Houston, TX, [2]EVA Exploration Architecture, EVA Management Office, NASA Parkway/FX, Houston, TX

Introduction: The first human mission to Mars will be the farthest distance that humans have traveled from Earth and the first human boots on Martian soil in the Exploration EVA Suit. The primary functions of the Exploration EVA Suit are to provide a habitable, anthropometric, pressurized environment for up to eight hours that allows crewmembers to perform autonomous and robotically assisted extravehicular exploration, science/research, construction, servicing, and repair operations on the exterior of the vehicle, in hazardous external conditions of the Mars local environment. The Exploration EVA Suit has the capability to structurally interface with exploration vehicles via next generation ingress/egress systems. Operational concepts and requirements are dependent on the mission profile, surface assets, and the Mars environment. This presentation will discuss the effects and dependencies of the EVA system design with the local Mars environment and Planetary Protection. Of the three study areas listed for the workshop, EVA identifies most strongly with technology and operations for contamination control.

Study Area 1, Microbial and Human Health Monitoring: It is assumed that most of the microbial and environmental monitoring related to particulates and backward contamination will be provided by the vehicle Environmental Control and Life Support System (ECLSS) and external tools, such as a detection kit. The suit includes sensors for pressure regulation, O2 pressure, CO2, and humidity levels. Other contaminants such as dust would not easily get inside the suit during an EVA with the bladder underneath the suit restraints being air tight, and the suit at an ambient pressure greater than the Martian atmosphere. Operations such as ingress, suit maintenance, etc. have potential to result in external dust contamination also within the vehicle/facility, not just the suit, as discussed under Study Area 2. This is where technology and operations for contamination control fit in.

Study Area 2, Technology and Operations for Contamination Control: Dust contamination and Planetary Protection must be treated with increased sensitivity on Mars. EVA is essential for exploration and a primary method for the efficacy of exploration mission success, research, and the goal of human pioneering. Each EVA performed on the surface of Mars allows dust to come into contact with the suits. Boots, gloves, elbows and knees will likely see the most accumulation of dust.

Layered Engineering Defense Plan: A Layered Engineering Defense Plan, which includes 6 layers, should be utilized to help mitigate the effect of dust on the suit materials, the transfer of dust on the suits, forward and backward contamination to the crew and habitation, cleaning and protection (interior and exterior) and the use of air quality contamination zones. [1]

The 1st layer includes materials and engineering design. Fabrication of an EVA suit with resistance to impact and abrasion from the Martian dust poses a significant engineering challenge. Technology advances are required for the material layup for both space and planet environments, and can include material exposure to dust, abrasion, punctures, cuts, hypervelocity impacts (micrometeoroids and secondary ejecta), and general vehicle materials compatibility without compromising suit mobility. [2] Dust tolerant mechanisms, seals, bearings, and electrical connectors are necessary to prevent connector shorts, and mechanical failure of connector. These particulate tolerant mechanisms should also be maintainable so that any dust/dirt/particulates can be periodically cleaned out. Pockets and folds should be minimized such that they do not collect particulates and sensitive equipment should include dust impermeable covers.

The 2nd Layer deals with operational controls, which includes eliminating, to the extent possible, suit ingress to the habitable volumes. Among multiple reasons that constitute the need to perform nominal EVAs from habitable elements, EVA space suit components have a limited life duration and must be maintained during a long duration human mission. The space suits need to be brought inside a habitable volume for nominal and contingency maintenance, which will introduce some amount of dust. The process will be a multi-phase integrated operation to limit dust introduction into the suits and into the crew cabin. ECLSS will provide air circulation and revitalization for contamination and particulate control in the cabin.

Operational controls also include ingress/egress methods that will mitigate dust transfer into the cabin, (i.e. rear-entry airlocks, suitport-airlocks, and suitports). Past methods of ingress/egress through airlocks would have the crewmember traversing/translating directly through the dust that was brought in after an EVA both after the crewmembers doff their suits and prior to donning their suits. A next generation airlock is needed to provide readily available EVA capability, particulate mitigation, and backward and forward plan-

etary protection by donning/doffing the rear-entry EVA suit through a bulkhead, such that the crewmember does not translate through the dust shirtsleeve during vehicle ingress and don/doff. To further prevent backward contamination, consideration is underway to leave the EVA space suits behind on the surface.

The 3rd Layer includes contamination prevention. Exploration of Mars must be conducted with planetary protection requirements, considering both forward and backward contamination prevention. Space suits inherently have some amount of venting/leakage. Human systems introduce the risk of forward contamination through venting of organics through water vapor with trace contaminants such as gases (CO, methane, etc.) and liquids (body oils, ointments, etc.) and leakage through flanges and fabric-to-metal attachment points. Some of the next generation airlock methods minimize the volume of consumables being vented from the vehicle; however, there is still gas during depress that is vented to the atmosphere. Vented gas as well as gas contained by reclamation systems can help reduce this with the proper amount of filtration in place in the vehicle's ECLSS. Likewise these same human systems introduce the risk of backward contamination to the crewmembers and to the Earth and need to be evaluated by human health and performance. Despite possible engineered controls designed within the suit and other assets, operational controls for planetary protection are anticipated to establish "keep out zones" or special regions prohibiting human presence in Martian areas deemed to be highly likely to contain life. These zones would probably be established by robotic precursor missions to conduct sensitive analyses before an EVA crewmember arrives. Flight rules and operational concepts need to be understood to work around potential special regions.

To the extent possible, Layers 4 and 5 are directed toward external and internal cleaning and will help minimize the amount of dust brought into a pressurizable area or ingress/egress method and minimize the cleaning of interior zones. Wipes (dry and wet), vacuum/cleaning systems and other potential cleaning tools that can safely remove and contain dust without exceeding the limitation of the space suit materials need to be evaluated. Contamination detection technology should be evaluated via purpose and operational concepts (hand-held vs. inside the ingress/egress method). Containment and cleaning technology needs to be evaluated. If sterilization is considered necessary in the ingress/egress method, it must not exceed the suit material limitations and must be compatible with the vehicle ECLSS and materials inside the ingress/egress method. When ingressing the chamber containing the space suits to perform suit maintenance, the crewmembers don Personal Protective Equipment (PPE) to the extent necessary, along with utilizing floor mats, pressure differentials, dust containment curtains, mud rooms, etc.

Finally, the 6th Layer includes air quality contamination zones. This is linked to engineering and operational design of the ingress/egress method as well as the overall asset architecture. As mentioned above, a conventional airlock would include a single volume for bringing the suit inside a pressurizeable volume for conducting suit maintenance. Next generation airlocks such as rear-entry airlocks/suitlocks and suitport-airlocks could include a secondary chamber or mud room to further contain contamination and increase air quality as the crewmember translates to the cleanest areas of the vehicles, such as habitats, pressurized rovers and ascent vehicles.

Strategic Knowledge Gaps for EVA: Strategic Knowledge Gaps include the need for an agency/program endorsed document that includes the following characteristics of the environment: chemical and physical properties of dust/dirt, particle size, shape, composition, toxicity, static electricity, electrical conductance, dust storms, etc. Guidance for use of dust simulants is needed for testing. If JSC-Mars 1 simulant is not adequate for testing mechanical systems, what is? [3] Do certain corrosive materials need to be added to the simulants for materials testing? What are the mitigation protocols? Do the dust properties change when exposed to a habitable environment (pressure, humidity, increased O2, etc.)? What type of hazards does the dust present to humans? A programmatic requirement for allowable levels of Martian contamination within the habitable volume is needed. Next generation airlock analogs/testing should be demonstrated to study levels of dust mitigation/planetary protection.

Closure of knowledge gaps can significantly increase the fidelity of early development testing of EVA suit materials and will help develop flight rules and operational concepts to enable more efficient human exploration of the Mars surface.

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LOW-LATENCY TELEOPERATIONS FOR MARS PLANETARY PROTECTION. M. L. Lupisella[1], M. Bobskill[2], M. Rucker[3], M. Gernhardt[4], B. Glass[5], [1]NASA Goddard Space Flight Center, Exploration Systems Projects, 8800 Greenbelt Rd., Greenbelt Md. 20771. Mark.L.Lupisella@nasa.gov. [2]NASA Langley Research Center, Space Missions Analysis Branch, 1 North Dryden Street, Hampton, VA. 23681, marianne.r.bobskill@nasa.gov, [3]NASA Johnson Space Center, Exploration Mission Planning Office, [4]NASA Johnson Space Center, Engineering Directorate, [5]NASA Ames Research Center, Intelligent Systems Division.

Introduction: Low-latency teleoperations has the potential to help address a number of planetary protection concerns associated with human exploration missions to Mars. Based on present conceptions of potential special regions on Mars, it may be prudent to find and explore such regions telerobotically in order to address contamination control concerns for those regions and to mitigate potential biohazards to crew members and possibly to terrestrial life when crew members return to earth. Depending on how special regions are ultimately defined and understood in their actual environments (e.g. through precursor mission data, modeling, etc.), and depending on what kinds of contamination control technologies may be implemented with crew assets (such as suits, rovers, sample acquisition systems, drills, etc.) it may also be prudent to explore these regions for long periods of time without humans in the immediate environment of interest. Similarly, if there is sufficient uncertainty about the biological or chemical nature of Mars samples, it may be wise to explore strategies for telerobotically conducting sample operations for long periods of time. Teleoperations may also be useful for addressing other operations related to planetary protection such as suit and other asset operations (e.g. cleaning, maintenance, etc.) and "end of life" mission operations.

The Human Spaceflight Architecture Team (HAT) Mars Destination Operations Team (DOT) examined these challenges from an integrated operational perspective and noted potentially significant roles for low-latency teleoperations (LLT – used interchangeably with 'teleoperations' and 'telerobotics' in this abstract). The HAT Mars Moons Team is also presently analyzing teleoperations from Mars orbit to assess trades, including potential advantages and implications of telerobotically exploring special regions from Mars orbit.

This presentation will provide background of science operations from the HAT Mars DOT 500-day surface operations concept work and focus primarily, although not exclusively, on 3 domains of Mars operations that are potentially relevant to planetary protection and LLT: (1) Mars orbit to Mars surface, (2) Mars surface location to Mars special regions, and (3) Mars science lab teleoperations.

Mars Orbit to Mars Surface Teleoperations: HAT is presently analyzing missions to the moons of Mars (and Mars orbit generally) prior to landing crew on the surface of Mars. One potential advantage of such missions is that orbit-to-surface teleoperations can be conducted with very short communication delays (e.g. well under .5 seconds). This kind of low-latency teleops can allow crew members to be highly responsive with surface assets on the Mars surface – a form of "real-time" or "near real-time" operations. These surface assets could be pre-deployed (prior to the human mission to Mars) or could be deployed during the human Mars orbit mission. Depending on the capabilities of those assets (e.g. mobility and scientific capabilities), it should be possible to scientifically explore fairly large areas of the surface, and possibly sub-surface, prior to crew landing on the surface. This could allow for previously unknown special regions to be found and for special regions in general to be directly explored thoroughly before crew lands on the surface. In this way, orbit-to-surface teleops can also potentially inform crew landing site selection.

Depending on what is learned about contaminant transport and contaminant impacts to sites of interest, a crewed surface landing may compromise planetary protection protocols. Crew operations of surface assets from Mars orbit would avoid this concern – as long as the telerobotic surface assets were properly cleaned and maintained. An outstanding question is the size of the area (including subsurface) that reconnaissance should cover and the kinds of analysis needed over that area prior to crew landing.

Drilling. Related to the above question, it may be preferable to drill for various reasons – ranging from resource prospecting to biohazard detection to the search for life. If drilling turns out to important, it will be one of the more challenging tasks and so will be an area of focus for this presentation. It may prudent to conduct drilling without having to put humans in the immediate drill zone, at least initially, if not for longer durations while sensitive drilling activities are being conducted. The following paragraphs are taken from a HAT drilling report on Mars drilling associated with human missions [2]:

A fully automated and/or telerobotic drilling system, with no hands-on human interaction, could meet planetary protection constraints but may not be practical, particularly for deep drilling operations which often require hands-on trouble-shooting. Depending on

the type of drilling technology selected, automation or telerobotic control could drive the need for specialized equipment manipulators, which in turn would require additional power and thermal conditioning. Adding to the complexity, subsurface sample collection and return to a Science Lab would also have to be fully automated or telerobotic.

A workshop on Planetary Drilling and Sample Acquisition held at the NASA Goddard Space Flight Center in May, 2013 noted significant technology gaps to overcome: "Automated core acquisition and handling, rugged and high-temperature sensor development and placement, automated drilling control software, and software testing and validation are technology gaps." The HAT drilling report built on this and made further recommendations:

Recommendation: If deep drilling operations must be autonomous and/or telerobotic due to planetary protection concerns, technology development emphasis should be placed on automated core and fluid acquisition and handling, low mass borehole stabilization, rugged and high-temperature sensor development and placement, automated drilling control software, and software testing and validation.

Recommendation: If deep drilling operations must be completely autonomous and/or telerobotic, a high fidelity mass and operational timeline analysis should be completed to determine whether it makes more sense to perform this activity on a crewed vs. robotic mission. Some of this analysis is underway and will be covered in the presentation.

Recommendation: Mass should be allocated for automated and/or telerobotic control for deep drilling systems (including power).

The time-scale for a drill to encounter difficulties is often on the order of 10 to 20 seconds, making teleoperation from Earth risky. Teleoperation from Mars orbit or Mars surface is feasible, but risk will vary with drill design and drilling conditions.

Mars Surface Location to Mars Special Regions: Much of what is conducted from Mars orbit via LLT could also be performed once the crew has landed on the surface. There is the additional potential advantage that for many scenarios the latency will be lower and potentially less complicated since surface assets may be closer and in direct line of sight at most (or all) times. The HAT Mars Destination Operations Team developed an integrated long-duration 500 day "science-driven" surface operations concept in which low-latency teloeperations was invoked as a possible strategy for exploring potential special regions while the crew is on the Martian surface at a safe distance from special regions. The team reviewed past literature and had invited presentations on planetary protection and contamination control. It was suggested that if the surface assets can be maintained to meet planetary protection protocols, then such assets could be teleoperated to safely explore potential special regions. It may also be that during the initial period of crew acclimation after the crew has landed, that LLT can be conducted from the habitat into the surrounding landing area in order to gain additional detailed understanding of the landing area environment before crew performs the first EVAs or other operations that may be problematic for contamination reasons.

Mars Surface Science Lab Teleoperations: It is also possible that the crew (either from orbit or from a surface location such as a habitat or pressurized rover), using telerobotic assets that are on the surface, could efficiently acquire samples and transport them to a predeployed science laboratory to conduct additional detailed analysis on the samples as needed. This additional analysis could be done via low-latency teleoperations of laboratory assets and would benefit from high-precision manipulators and advanced scientific instruments. Similarly, once the crew has landed, a similar strategy can be used when the crew is on the surface since this will allow samples to be relatively isolated from other surface assets and their associated operations (e.g. such as crew habitats).

Additional Considerations: Cleaning and maintenance of surface assets such as suits and rovers is likely to be a key operational activity. Telerobotics has the potential to provide an effective way to safely perform complex tasks of this kind without exposing the crew directly to Mars material or vice-versa. It may also be worth considering potential "end of mission" concerns and the associated operational details such as disassembly applications or "seal up" operations (e.g. hab, rovers, etc.) that may be best done telerobotically either when crew on the surface or from Mars orbit before the crew leaves the Mars system. Disposing of the Mars ascent vehicle (MAV) could be a unique challenge because impacting it somewhere could release contaminants. The MAV could be teleoperated to a state that meets planetary protection requirements (e.g. venting down to vacuum to try to kill organisms, transport to, and/or orientation in, a safe in-space location, or possibly a "controlled" landing to a safe zone if needed).

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MARS SAMPLE RETURN FEEDFORWARD OF POTENTIAL PLANETARY PROTECTION TECHNOLOGY/KNOWLEDGE TO HUMAN EXPLORATION. L. E. Hays[1], D. W. Beaty[1], and M. A. Jones[2]. [1]Mars Program Office, Jet Propulsion Laboratory, California Institute of Technology, Pasadena, CA 91109 (lindsay.e.hays@jpl.nasa.gov), [2]Biotechnology and Planetary Protection Group, Jet Propulsion Laboratory, California Institute of Technology, Pasadena, CA 91109.

Introduction: At our current vantage point, two major potential enterprises lie in the future: Mars sample return (MSR), and human exploration of Mars. Although many significant planetary protection-related knowledge gaps are associated with the latter, there is significant overlap in the type of research that would help address current planetary protection knowledge gaps for both lines of discovery. It is therefore important that these overlaps are identified early, so that information that addresses these knowledge gaps is shared in order to minimize duplicating effort and maximize achievement of common goals. In these areas, the expected accomplishments of potential sample return would not need to be separately planned and budgeted for in association with a human mission. In this paper, we plan to introduce for discussion two topics where information from MSR could clearly feed forward into human extraterrestrial missions, and one topic where the path to sharing information is less clear (and perhaps not possible).

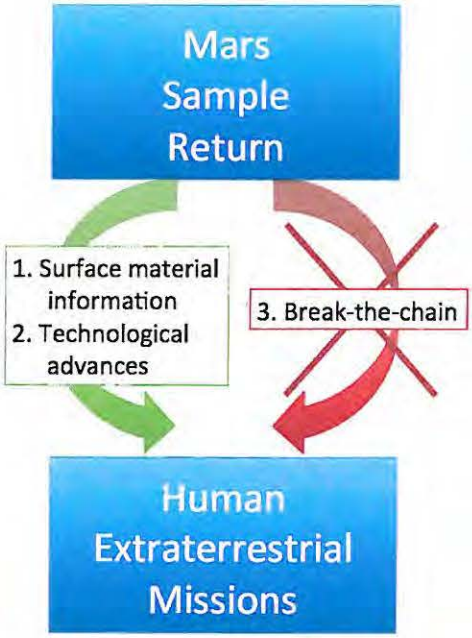

Figure 1. General overview of the feed forward of knowledge from Mars sample return to human extraterrestrial missions.

Areas of Overlap: There are at least two general aspects of planetary protection planning and implementation strategy related to the multiple possible missions of the MSR campaign that would benefit human exploration:

1. Detailed analysis of martian surface materials.
2. Technological advances in cleaning and contamination control.

Martian surface material analysis: The primary planetary protection question that could be addressed by potential MSR analyses would be results of investigation into the potential for past or present life on Mars. In addition, returned samples could also provide detailed information about the physical, chemical, and electrical properties of the martian regolith – strategic knowledge gaps which were acknowledged in the 2012 P-SAG final report [1]. In that report, knowledge gaps were considered with respect to the direct effects on astronauts for a human mission. However, they are also relevant specifically to planetary protection in that they provide the detailed information needed to make specifications for meeting requirements pertaining to cleaning, sterilizing and preventing re-contamination in surface habitat environments.

Questions addressed: If no martian life is detected in returned samples, but human extraterrestrial missions explore a different location on Mars, what environmental information from returned samples could be useful to minimizing human impacts in the explored location? How can information about returned martian regolith be used to study the effect of dust on seals designed to minimize contamination?

Technological advances: The second area of overlap between MSR and human extraterrestrial missions would be in the technological advances developed for controlled recovery and analysis of the returned martian samples. These technical advances could then be used for developing in-situ cleaning and sterilization protocols for nominal surface operations of a human mission, or for back up clean-up protocols in the event of an inadvertent release of terrestrial material, among other applications. If considerations for the type of developments needed for human exploration were outlined within the MSR timeline, these technological developments could be broadened to meet both needs.

Questions addressed: How can methods designed for and tested on returned samples be used to design effective cleaning methods for nominal operations during human exploration? How can studies into clean-up of the location where the potential returned sample container would impact Earth's surface, combined with information about physical properties of returned martian regolith, be used to design environmental clean-up protocols for Mars surface operations?

In addition to feeding into general operations of any future human extraterrestrial missions, information from Mars sample return could also inform decisions that were made as to the type of science investigations that future astronauts could perform without risk of contamination of the martian surface or risk to themselves.

Break-the-chain: Although breaking the chain of contact with Mars for sample return is a familiar, though complicated, problem, for human exploration of Mars there may conceivably be no way to break the chain of contact if an astronaut becomes part of the "chain." Although Mars sample return technologies can help minimize the chances that this scenario would occur, if it did, the type of break-the-chain architecture developed for MSR may not be applicable to human missions.

Questions addressed: Is perfect separation between the martian surface environment and human surface habitats possible? Are there break-the-chain options that exist or could be explored for human exploration?

Conclusions: Although there are certainly areas of overlap between Mars sample return and human extraterrestrial missions where data addressing knowledge gaps from the former could be fed forward into similar knowledge gaps in the later, many of these focus on human health factors and questions of design of surface habitats and mobility systems. When considering the overlaps that are of concern to planetary protection, although there are fewer, some of the remaining questions can only be answered by sample return. Although feed-forward of information from Mars sample return could be of significant value to human exploration, in order to most efficiently use the very valuable resource of returned samples, the planetary protection investigations undertaken must be carefully planned well in advance.

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MARS SOIL-BASED RESOURCE PROCESSING AND PLANETARY PROTECTION. G. B. Sanders[1] and R. P. Mueller[2], [1]NASA Johnson Space Center, 2101 NASA Parkway, Houston, TX, gerald.b.sanders@nasa.gov, [2]NASA Kennedy Space Center, FL, rob.mueller@nasa.gov

Introduction: The ability to extract and process resources at the site of exploration into products and services, commonly referred to as *In Situ* Resource Utilization (ISRU), can have significant benefits for robotic and human exploration missions. In particular, the ability to use *in situ* resources to make propellants, fuel cell reactants, and life support consumables has been shown in studies to significantly reduce mission mass, cost, and risk, while enhancing or enabling missions not possible without the incorporation of ISRU. In December 2007, NASA completed the Mars Human Design Reference Architecture (DRA) 5.0 study [1]. For the first time in a large scale Mars architecture study, water from Mars soil was considered as a potential resource. At the time of the study, knowledge of water resources (their form, concentration, and distribution) was extremely limited. Also, due to lack of understanding of how to apply planetary protection rules and requirements to ISRU soil-based excavation and processing, an extremely conservative approach was incorporated where only the top several centimeters of ultraviolet (UV) radiated soil could be processed (assumed to be 3% water by mass). While results of the Mars DRA 5.0 study showed that combining atmosphere processing to make oxygen and methane with soil processing to extract water provided the lowest mission mass, atmosphere processing to convert carbon dioxide (CO_2) into oxygen was baselined for the mission since it was the lowest power and risk option [2]. With increased knowledge and further clarification of Mars planetary protection rules, and the recent release of the Mars Exploration Program Analysis Group (MEPAG) report on "Special Regions and the Human Exploration of Mars" [3], it is time to reexamine potential water resources on Mars, options for soil processing to extract water, and the implications with respect to planetary protection and Special Regions on Mars.

Potential Water Resources for ISRU: The concentration, form, depth, and distribution of water resources on Mars varies greatly as a function of latitude and geological features. At the equatorial region (between 30°S and 30°N), areas of enhanced water from Mars Odyssey neutron analysis are usually interpreted as being due to hydrated minerals. These minerals can contain water at extremely low concentrations <2% to as much as 13% by mass. At the mid latitudes (30° to 60°) subsurface ice/permafrost may exist in the top 5 meters and fresh impacts have exposed ice excavated from 0.3-2.0 meters in depth. At higher latitudes, ice exists at higher concentrations and closer to the surface, but are seasonally covered with CO_2 ice in the winter. Soil excavated by the Phoenix lander descent and landing rocket plume and the robotic excavator arm showed dirty near-pure ice near the surface. Two other forms/location of water on Mars have been postulated but not yet proven; subterranean aquifers and briny water in Recurring Slope Lineae (RSL). Since both of these potential forms of water are considered Special Regions, they have been excluded for consideration as resources for ISRU extraction operations. Both hydrated minerals and icy soils are considered viable resources for ISRU extraction operations.

Excavation and Soil Processing Techniques for Water Extraction: Two general approaches to extract water from Martian soil have been considered: remove the water *in situ* from the soil, and excavate and transfer the soil to a heating chamber that can be enclosed so that water released can be removed and collected.

To remove the water in situ, the surface of the soil to be processed needs to be covered with a collection dome and the soil heated directly. Two concepts have been proposed to heat the soil directly; 1) via solar heating through a greenhouse-like transparent dome and 2) via microwave energy. Once the soil is heated, water is released in the form of vapor and is collected in a cold trap. Due to the low heating rate from solar energy, the rate at which water is evolved will be low. Also, because of the significant potential for the water vapor to recondense in the soil or other locations than the cold trap, no significant work has been performed to advance this method of water extraction. Experimental work has been performed using microwaves to extract water from ice in lunar regolith simulants that shows promise [4]. Because microwave energy can be concentrated, the collection dome can be much smaller. To promote the evolution of water, it has also been proposed to drill holes into the soil before heating to provide a path for water vapor migration to the cold trap. While this approach for *in situ* water extraction will be much more efficient than solar heating, there is still the potential for water vapor condensation back into the soil at the border between heated and non-heated soil thereby reducing extraction efficiency.

To excavate Mars soil for subsequent processing, the technique chosen will have to consider the soil and form of water present (hydration vs icy) and the compactness/hardness of the soil. For hydrated soils or

soils with low concentrations of ice, traditional excavation methods such as the lift-haul-dump or bucketwheel/bucketdrum concept may be favored. As ice concentration increases, along with material hardness, vibratory blades or augers to break up and move the material may be required. Once the material has been excavated, it can then be transferred to a heating chamber. Three heating methods have been considered for soil processing; 1) electrical heaters/conduction, 2) fluidization/convection, and 3) microwave heating. Significant work has been performed over the last 10 years on the first two heating methods for hydrogen reduction of lunar regolith, lunar ice prospecting, and Mars soil drying [5]. To remove all the water from hydrated soils typically requires the soil to be heated to above 600°C. However, based on data from the Sample Analysis on Mars (SAM) instrument on the Curiosity rover, a heating limit of <450°C may be desired to mitigate the production of HCl and H_2S contaminates released as carbonates and perchlorates in the soil breakdown as temperature increases [6]. A significant fraction of the water (~80%) is released below 450°C. Laboratory tests suggest heating times of 30 to 60 minutes may be required. For removal of water from icy soils, the heating temperature is expected to be <300°C. An interesting concept that combined using an auger to excavate material and an enclosure cap to heat the soil while still on the auger blades was designed and built by Honeybee Robotics, called the Mobile In Situ Moon/Mars Water Extractor (MISME). The MISME concept demonstrated that significant amounts of water could be obtained from icy soils with minimal hardware and energy [7]. Once the soil is processed, it will be removed from the heating chamber and dumped back on the ground; either immediately or delivered to a designated site.

Soil-based ISRU and Planetary Protection: Any soil-based ISRU process on Mars needs to ensure that i) there is no Forward Contamination from the hardware utilized, and ii) no Special Region is created based on the excavation and processing of soil containing water. To ensure no forward contamination occurs, the ISRU excavation and soil processing hardware will be sterilized to Viking mission standards before launch. This is not considered to be a significant design issue for the soil heating chamber since sustained operating temperatures of up to 450°C are expected. COSPAR defines Special Regions as "a region within which terrestrial organisms are likely to replicate" and states that "any region which is interpreted to have a high potential for the existence of extant Martian life forms is also defined as a Special Region" [8]. It is therefore important for ISRU process not to allow water in Martian soil to remain in a liquid state for an extended period of time. The duration of this time is TBD and will need to be agreed upon with the Planetary Protection community. Therefore, soil processed to extract water may be required to be cooled below the freezing point before discarding back to the surface. Processing durations may also be imposed on methods associated with in situ heating of the Mars soil as well.

Conclusion: The processing of the soil on Mars to extract water for subsequent use in making propellants, fuel cell reactants, and life support consumables is enabling for future human exploration of Mars. The processing of Martian soil to extract the water present raises both Forward Contamination and creation of Special Region issues that will need to be addressed before operations can begin. At this time, it is believed that the currently proposed Mars soil-based ISRU concepts will be able to mitigate both of these planetary protection concerns.

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MITIGATING INADVERTENT CONTAMINATION IN SUBSURFACE DRILLING. B. Glass[1], G. Paulsen[2], K. Zacny[2], A. Dave[1]. [1]NASA Ames Research Center, Moffett Field, CA 94305, USA, Email: brian.glass@nasa.gov, [2]Honeybee Robotics, Pasadena, CA, 91103, USA.

Introduction: The issue of drill bits or other sampling mechanisms penetrating a subsurface Special Region is a current sampling technology gap in planetary protection. Our concept is to develop and test a new method of subsystem sterilization (bit sterilization) compatible with sample retrieval and transfer to in-situ spacecraft instruments or for sample return caching. With the drill bit's internal-heater self-sterilizing capability, this concept also tackles how we could recover during a mission if an accidental contamination does occur.

With this advance in planetary protection technologies and practices, the accidental contamination of a drill during sample transfer (or wind-blown particles off a spacecraft deck, or other vectors) would not necessarily mean a difficult choice between end-of-mission or else introducing potential contaminants into a Special Region.

Background: Our team have also participated in a Discovery-class mission proposal, called "Icebreaker", (Figure 1) which is a Phoenix-derived Mars polar lander with life and organics detection instruments and a 1 m sampling drill (McKay 2013). As a solar system exploration mission, the Icebreaker mission must comply with the Planetary Protection requirements established by NASA policy NPD 8020.7G and detailed in NPR 8020.12D, "Planetary Protection Provisions for Robotic Extraterrestrial Missions."[1].

The Phoenix mission to Mars was considered a Category IVc mission because the arm on the lander accessed a Special Region – the subsurface ice. As a result the arm was sterilized to the IVc requirements [2,3] and the rest of the spacecraft was cleaned to IVa requirements. To ensure that the arm remained sterilized the arm was encased in a biobarrier cocoon [2] during assembly and deployed from the cocoon (with some difficulty) on Mars.

Figure 1. Concept of "Icebreaker" Phoenix-derived polar drilling lander

Mars polar drilling and sampling missions (like Icebreaker) will access the subsurface ice. If this ice is still considered a Special Region, the planetary protection requirements for Icebreaker will be the same as for Phoenix.

1. The main part of the spacecraft will need to satisfy Category IVa cleanliness.
2. The drill and any portions of the spacecraft that could come in contact with the ice in the subsurface will need to satisfy Category IVc requirements.
   - *sterilization*
   - *biobarrier containment*
   - *non-contact with unsterilized lander components during operations*

Current practice for sterilization for Planetary Protection purposes uses dry heat microbial reduction (DHMR). This is a NASA certified process [4, 1] which involves temperatures in the range of 104 to 125°C with controlled absolute humidity, for durations that depend on the temperature. Barengoltz [5] points out that DHMR may be used without any assay and with the surface spore burden density specifications, or with a prior assay to establish a lower pretreatment density. Biobarrier containment was a significant challenge for the Phoenix arm [2] and will be a challenge for the Icebreaker drill. Any sample handling subsystem that is in contact with the drill will also have to be included within a biobarrier. On Mars, the drill assembly will have to emerge from the biobarrier to commence operations.

Approach: Automated sample delivery while breaking the chain of contact (maintaining sterile/nonsterile separations from a drill) is an unresolved technology gap that must be addressed before astrobiology missions can penetrate Category IVc (Special Region) areas. Our drilling planetary protection concept proposes to develop prototypes and test two aspects of planetary protection of the subsurface: a robotic system for retrieving and conveying drill or scoop samples to instruments, without drill contact; and a new planetary-protection technology: an embedded heat-sterilization inductive heating loop inside the drill bit, capable of re-sterilizing the drill in case of

unplanned or inadvertent contact with the sample handling system (or other sources of contaminants). Of interest for study are the microbial counts before and after sterilization protocols, allowing for iterative improving of protocols and designs based on the contaminant reduction performance of these two approaches.

Our conceptual approach is to reduce spore counts by heating the drill in-situ. While a few minutes of high surface temperatures is not equivalent to Viking-standard long-duration/modest-temperature baking (~120°C x 24-48 hours), it will still reduce and alleviate spore counts blown over or from inadvertent contact with a "dirty" spacecraft.

In terrestrial exploratory drilling where bio-cross-contamination is an issue, current practice after sample acquisition is to clean and then reduce the bio-load of the drill end before re-insertion into a borehole. Under field conditions on Earth, this is typically done to exploration drills by using strong solvents and/or by dousing the drill string in alcohol and setting it on fire briefly. Heating would occur outside the borehole, and the drill string would then be expected to loiter after a heat-disinfection cycle sufficient to return to surface ambient temperatures prior to reinsertion.

As a side benefit, in an emergency an embedded bit heater could reduce mission-loss risks significantly -- by providing a means of freeing a drill string frozen stuck in an ice layer. And the bit-end temperature sensor could also be used for downhole heat-flow measurements. Starting with just an initial concept here, it would be possible to demonstrate the effectiveness of an embedded heater in a drill bit by integrating commercial off the shelf (COTS) components with existing (Icebreaker) or new drill hardware. In the design example shown in Figure 2, a 28.6 mm (1.125 inch) diameter COTS drill bit is shown with a 200 W and 871°C (1600°F) capable COTS Cartridge Heater with internal type K thermocouple. The COTS drill bit and heater/thermocouple combo are shown integrated with an existing auger design.

While there is a significant spacecraft power draw for a bit heater, it would be only about 2-3 times the load of normal drilling operation levels (70-100 W), and heated periods are expected to be much shorter than the time spent drilling. This fits within expected spacecraft drilling power budgets. If it proves to be an effective in situ sterilization approach, it could conceivably reduce or eliminate the need for DMHR/biobarrier for the bit assembly.

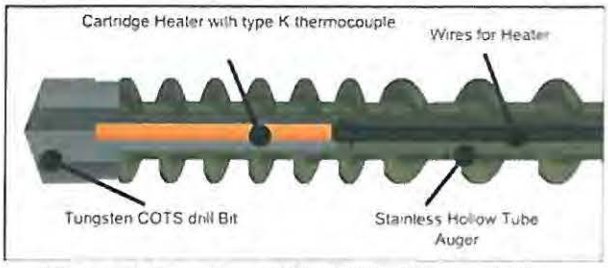

Figure 2. Location of Cartridge Heater in Icebreaker-Compatible Drill Bit.

Technology Objectives and Maturity Goals: Two key goals of this project will be to (1) assess the effectiveness of the heated drill bit to sterilize the exterior surface of the drill prior to penetrating the subsurface, and (2) to characterize the extent that drill cuttings and subsurface dusts might contaminate the drill rig, payloads, and local terrain. The 1st goal is to prevent the forward contamination of the subsurface, and the 2nd goal is to prevent the back contamination of the drill rig and terrain. We will seek to improve the readiness of planetary protection and contamination management technologies, demonstrated in both Mars chamber environments and analog-site field tests. Field tests are necessary to provide confidence in robustness, force system stand-alone integration, and to discover any unanticipated design flaws that were masked by the constraints of small test chamber sample targets.

Conclusion: A new sample acquisition planetary protection concept offers promise through the development and testing of a new method of subsystem sterilization (embedded bit heater) compatible with subsurface sample acquisition and transfer.

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Title: Case Study of Human Flora and Spore Contamination

Staying ahead of the curve in regard to Environmental Monitoring requires an understanding and an anticipation of the constantly changing microbial climate of the pharmaceutical manufacturing process. Knowing the characteristics of the environments in that process provides the benefit of allowing us to predict and solve potential future microbial control issues. This session will discuss pathways to use the data obtained from environmental monitoring to provide proactive and practical solutions for comprehending and managing everyday pharmaceutical microbiology challenges. This presentation will cover case studies on human flora and spore contamination in cleanroom operations. Solutions will be discussed to proactively present future contamination issues.

The seminar will cover the most common causes of contamination: operators, items brought into the cleanroom, and degradation of the cleanroom over time. Case studies in operator borne contamination will be discussed as well as preventative long term solutions. Specific examples of aerobic and anaerobic vegetative bacteria will be highlighted and analyzed. Items brought into cleanrooms that can harbor fungal and bacterial spores will be discussed as well as specific case studies highlighting examples where sources were items brought into the cleanroom or in some cases other common causes. Fungal and bacterial spore structures as well as efficacy testing and specific test conditions will be briefly discussed. Solutions will be presented to prevent efficacy testing failures due to test method, recovery, and coupon porosity issues. Targeted long term solutions will be discussed regarding the spore case studies in a concerted effort to limit reoccurrences.

The overall objective and scope of this seminar will be to discuss specific cases studies that have occurred in my years of experience in the industry. Specific and precision long term solutions will be conveyed to prevent reoccurrences and yield higher levels of control in the cleanroom operations.

SAMPLE CONTAINMENT TECHNOLOGY FOR MARS SAMPLE RETURN. Bob Gershman, JPL, 4800 Oak Grove Drive, M.S. 321-520, Pasadena, CA 91109. robert.gershman@jpl.nasa.gov.

The U.S. National Research Council and its European counterpart have considered the possible risks to the Earth's biosphere represented by potential Mars sample return (MSR). They recommended that "Samples returned from Mars by spacecraft should be *contained* and treated as though potentially hazardous until proven otherwise." NASA procedural requirements (NPR 8020.12) specify that the mission and the spacecraft design shall provide a method to "break the chain of contact" with Mars. All Mars material delivered to Earth must be inside a robustly sealed container, and no uncontained hardware that contacted Mars may be returned to Earth unless sterilized. The NASA Planetary Protection Officer has provided a draft "containment assurance" requirement for the first conceptual MSR mission: $<10^{-6}$ probability of inadvertent release of a single unsterilized Mars particle to the Earth's biosphere.

This talk will describe technology NASA is developing to meet the likely containment assurance requirement for the potential robotic MSR campaign. This includes methods for sealing the sample container with extremely low probability of external contamination, either on the Mars surface or in Mars orbit, and methods for sterilizing any residual contamination. Sealing modalities being investigated include brazing, explosive welding, bagging, and conventional o-rings. Sterilization modalities include heat, pyrotechnic paint, plasma, and hydrogen peroxide; but it should be noted that NASA has not yet considered which of these – if any – could be certified for sterilizing Mars material. Also, technology is needed to assure (with an unprecedented degree of confidence) that the Earth entry vehicle would withstand the thermal and structural rigors of Earth atmosphere entry and that the sample container and its seals would survive Earth entry, descent, and landing. Concepts for a new Earth entry vehicle that could satisfy the stringent MSR reliability requirements have been under study for several years, including some preliminary technology development activities.

UNDERSTANDING THE PROCESS AND DRIVERS FOR DEVELOPING HUMAN EXPLORATION PLANETARY PROTECTION REQUIREMENTS. M. A. Jones[1], D. W. Beaty[2], and L. E. Hays[2], [1]Biotechnology and Planetary Protection Group, Jet Propulsion Laboratory, California Institute of Technology, Pasadena, CA 91109 (melissa.a.jones@jpl.nasa.gov), [1]Mars Program Office, Jet Propulsion Laboratory, California Institute of Technology, Pasadena, CA 91109.

Introduction: The Planetary Protection Subcommittee of the NASA Advisory Council (NAC) recognized and recommended in May 2012 that a NASA Procedural Requirement should be developed to handle planetary protection requirements specific to proposed future human missions as a parallel document to 8020.12, Planetary Protection Provisions for Robotic Extraterrestrial Missions [1] under NASA Policy Directive 8020.7, Biological Contamination Control for Outbound and Inbound Planetary Spacecraft [2]. Between May 2012 and March 2013 the full NAC endorsed the recommendation and the NASA Administrator subsequently agreed [3]. However, the fact remains that there is insufficient knowledge in both scientific and technological domains to be able to set detailed planetaery protection requirements to the level that has been done for robotic missions in 8020.12. Therefore, NASA Policy Instruction (NPI) 8020.7, NASA Policy on Planetary Protection Requirements for Human Extraterrestrial Missions, was developed, which describes general policy guidelines and approaches as a placeholder until enough information is determined to generate a full requirements document (NPR) [3]. COSPAR also amended its original 2002 policy regarding proposed future human exploration of Mars, most recently in 2011; NASA missions are required to be consistent with this policy [3].

Getting to requirements: Fundamentally, requirements must be rooted in policy but also consider the current state of affairs on the target body as well as engineering realities. An approach to developing planetary protection requirements for human exploration would be to take a "systems view" approach to try to ensure all necessary requirements are accounted for across the "system." Those requirments can be flowed down through the required levels with an outcome of clearly implementable requirements to be met by the science and engineering team on the mission. This approach of using a typical systems engineering process for flow down of planetary protection requirements has been implemented recently on an upcoming Mars robotic mission with resounding success so far, and there are plans to take a similar approach on other missions. Therefore, it makes sense to advocate for utilization of a similar "systems view" approach for the development of planetary protection requirements for future human exploration.

In order to advance to actually developing requirements, it is often valuable to take key drivers into account, whether it be requirement drivers or process drivers. The paragraphs below describe some of the potential drivers that should be recoginized and discussed while addressing planetary protection requirements development for human missions.

Potential policy changes: According to the COSPAR Policy and Guidelines for Human Missions as denoted in Attachment A of NPI 8020.7, "The intent of planetary protection policy is the same whether a mission to Mars is conducted robotically or with human explorers. Accordingly, planetary protection goals should not be relaxed to accommodate a human mission to Mars. Rather they become even more directly relevant to such missions – even if specific implementation requirements must differ" [3]. The intent of the policy—the protection and preservation of the body being investigated as well as our home planet—is not likely to be arguably different between a robotic or human mission. However, has the feasibility of implementing similar robotic contamination requirements on a human mission been investigated in at least a bounding case format to fully understand the drivers? What are the limits of implementation for future proposed human missions versus robotic missions, potentially including resources? Is there potential that human exploration-related policy might drive changes to the robotic policy? For example, could there be changes in the level of contamination allowed, perhaps in specific areas of Mars? Would this level of investigation point to other noteworthy knowledge gaps?

Knowledge development: NPI 8020.7, Section 4, describes a set of study areas that are critical to obtain the information to proceed forward on developing requirements for human missions, with community input being sought for additional areas. The driver in this area is likely to be those items related to understanding environmental processes on Mars and other bodies.

While modeling capabilities have gotten better over time, scientist still depend on data from the body itself to understand and validate modeling efforts. To date, it has taken several decades of multiple Mars missions to get to where we are today, and perhaps (arguably) there is still a lot to learn to be able to understand even "transport and sterilization of organism released by human activity" on Mars [3]. How much data would

need to be collected from any notional human landing site, and how much needs to be collected from Mars in general? Data from the actual landing site is perhaps the best, but this would require making a final selection of a landing site 1-2 decades in advance of the launch. Therefore, a critical driver in developing policy, in particular requirements, may be determining what missions and experiments are necessarily performed at the target body, which may take many mission and decades to complete. This could place a lot of activity on the critical path that must be addressed by robotic precursors leading up to a future human mission. In addition, there is the question of what policy guidelines might need to be in place if we do not get the answers deemed necessary to proceed with a future human mission.

A roadmap for the path forward: NPI 8020.7, establishes the policy guidelines and describes the approach for obtaining the information needed over the "next few years" to draft an equivalent NPR for human missions [3], which includes a "path forward" section outlining the roadmap through which the NPR will be developed. It seems advantaegous and even critical that the process is started now, so that the requirements are ready when needed. However, given that there are likely to be several critical path items and schedule drivers (e.g., robotic precursor missions, required process steps, technology development needs), it might be a useful exercise to work the time problem backwards, given more resolved understanding of critical inputs and a given target goal date, to determine feasibility of developing a implementable set of requirements in an NPR in the next few years. It may emphasize schedule drivers, such as places where processes and technology development may be useful to prove out or develop on precursor missions (e.g., could the proposed Asteroid Redirect Robotic Mission be a "proving ground" for future humans to Mars missions?) and where development of some specific sections of the NPR may be well ahead or potentially lag behind, for some particular reason.

Conclusions: While work has begun to develop the necessary planetary protection requirements for proposed future human space exploration, particularly for Mars, through COSPAR policy ammendments, recommendations by the Planetary Protection Subcommittee, and development of NPI 8020.7, it is beneficial to take a systematic end-to-end approach ("system view") to determine the best path forward for planetary protection requirements development for future human missions. It is critical to determine as early as possible the driving factors (including some potential ones discussed above), and answer question about them as quantatively as possible for development of the process as well as the actual requirements. The advantage is that this would typically ensure a robust, stakeholder-supported process for developing a clear and implementable NPR detailing planetary protection requirements for proposed future human exploration.

References: [1] NPR 8020.12D, Planetary Protection Provisions for Robotic Extraterrestrial Missions (See NASA NODIS Library for current document). [2] NPD 8020.7G, Biological Contamination Control for Outbound and Inbound Planetary Spacecraft (See NASA NODIS Library for current document). [3] NPI 8020.7, NASA Policy on Planetary Protection Requirements for Human Extraterrestrial Missions (See NASA NODIS Library for current document).

FORWARD PLANETARY PROTECTION ISSUES AND CONSTRAINTS RELATED TO PLANNING FOR THE POTENTIAL HUMAN EXPLORATION OF MARS. D. W. Beaty[1], R. M. Davis[2], V. E. Hamilton[3], L. E. Hays[1], M. A. Jones[1], D. S. S. Lim[4,5], J. D. Rummel[6], and R. Whitley[7]. [1]Mars Program Office, Jet Propulsion Laboratory, California Institute of Technology, Pasadena, CA 91109 (dwbeaty@jpl.nasa.gov), [2]NASA HQ, Washington, DC, [3]NASA Ames Research Center, Mountain View, CA 94035, [4]BAER Institute, Petaluma, CA 94043; [6]East Carolina University, Greenville, NC 27858, [7]NASA Johnson Space Center, Houston, TX 77058.

Introduction: A human mission to the martian surface would potentially constitute a large biological contamination event for at least one location on Mars, and depending on the design of the mission, possibly more than one. In order for such a mission to be accomplished in an acceptable manner, the planetary science community needs to debate and consider the best answers to the following broad questions:

1) How can a human mission to Mars limit its potential contamination of the planet?
2) Where are the places on Mars where a large biological contamination event would be acceptable, if it were to occur?
3) Would a human mission entail a lifting, either partially or entirely, of the restrictions on the biological contamination of Mars?

At the present time, a primary driver behind the restrictions on "forward" contamination is that the exploration of Mars is in an active phase of the search for evidence of indigenous life (both past and present). As we look ahead to a possible human exploration program focused on Mars, there are three logical ways the future could unfold: At the time of human landings on Mars, 1) Mars is still in a period of biological exploration, and restrictions on Earth-sourced biological contamination are still essential to scientific success; 2) Biological exploration of Mars has been completed without detecting life, and restrictions on biological contamination are no longer necessary for that purpose; and 3) (which can be paired with either of the other two) Restrictions on biological contamination are essential to prevent damage to resources on Mars capable of supporting human colonists.

The Concept of Special Regions: Special Regions on Mars are defined as places (3D volumes) within which terrestrial microbial life could take a foothold, prosper, and reproduce. Because this would have the potential to confound the scientific exploration for indigenous life on Mars, a high priority requirement for spaceflight missions is to avoid such contamination, keeping the Special Regions safe (for additional details, see Rummel et al., 2014, and references therein).

Locational Constraints on a Human Landing Site: We anticipate that a human landing site will need to have some degree of spatial separation from Special Regions in order to avoid deleterious contamination effects. Currently, however, we don't yet have a good way to establish the scale of that separation, taking into account wind, dust storms, and potential subsurface connectivity. Until this separation can be quantified for a particular landing site/Special Region combination, it is not known how close a source of contamination can be allowed to get to a previously identified Special Region. This issue has previously been recognized by MEPAG, and it is discussed in the MEPAG Goals Document (most recent version: MEPAG, 2014).

Relationship to MEPAG Goal IV: In the MEPAG Goals Document, the importance of protecting Special Regions from human-sourced contamination is described in Investigation IV-2B. MEPAG points out that it is logically necessary to know, in advance of human missions, not only where the Special Regions are located (including those formed by natural processes, but also those that could be induced by some element of the human mission), but also the pathways and probabilities for the transport of contaminants to a nearby Special Region. Investigation IV-2B specifically calls for "understanding the rates and scales of the martian processes that would allow for the potential transport of viable terrestrial organisms to Special Regions." ". Once the Special Regions are located, we would need this to determine how close, and under what circumstances, human-related contamination could be allowed.

A practical question that needs discussion is what are the necessary informational inputs to determining the rates and scales of the various processes that would be relevant to the contamination of Special Regions? . This would need to include assessments of the form and probable quantities of the biological contaminants associated with human surface operations, such as that:

- discharged into the air,
- deliberately buried (either encapsulated or not),
- adhering to the surface of equipment/spacesuits that are exposed to the martian environment
- other.

We would then need to assess the factors that relate to the mechanical dispersal of these biological contaminants, including speed, direction, and duration of the winds, adhesion coefficients of microbes/particles under martian conditions, etc.

Finally, we would need to understand the lethality of the martian environment to Earth-sourced microbes as a function of time and space. As the contaminant plume spreads, the live organisms would be affected by UV radiation, dessication, oxidation, lack of nutrients, etc., which would cause the live organisms to die (but not disappear—the dead remains would still be part of the contaminant plume).

What do we need to measure or model to reach a community-acceptable solution to the above questions? At least some of these inputs can be obtained from experiments and models here on Earth, but are there also data sets that would need to be collected from Mars? If so, this requires careful planning through NASA's robotic Mars Exploration Program.

The inverse of Special Regions: If we presume that future exploration of Mars leads—at some point—to relaxing the restrictions on biological contamination carried by spacecraft—in particular regarding the allowable level of risk with respect to the contamination of at least parts of Mars—there may be in the future identifiable portions of Mars that are functionally the inverse of Special Regions. Instead of places where less than the current levels of spacecraft contamination (which have heretofore applied only to robotic missions) are allowed, there could be places on Mars where the additional contamination associated with human habitats and spacecraft would be allowed. Two key questions about implementing this potential future would be 1). What is the process whereby these places are identified and vetted, and 2). What is the timing wherein this process takes place?

Whatever the case, it is not within the planning horizon that we will remove all restrictions on biological contamination on Mars. In fact, there are many restrictions in place (but less than fully adequate) on the transport of biological contamination on Earth [e.g., 4]. We can envision that Mars will have certain sites (such as Special Regions and other areas where Earth organisms are not allowed) far into the future, and we can hope that the process and timing by which international PP policy would be revised relative to human missions will consider the situation carefully as the exploration of Mars continues.

Conclusions: The above issues are significant input to planning for the specific location (or locations) to be considered for human exploration activities on the surface of Mars. As of this writing, momentum seems to be increasing, in more than one sector here on Earth, for crewed missions to the Red Planet, both for exploration and for long-term habitation. It would be prudent to start now in discussing a process whereby the technical needs and timing associated with the biological exploration of Mars, human exploration interests, and our internationally basedother international planetary protection provisions can be simultaneously satisfied.

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Planetary Protection Knowledge Gaps for Human Extraterrestrial Missions, Ames Research Center, Moffitt Field, CA;
March 2015

ULTRAVIOLET IRRADIATION ON THE SURFACE OF MARS: IMPLICATIONS FOR EVA ACTIVITIES DURING FUTURE HUMAN MISSIONS. A. C. Schuerger, University of Florida, Space Life Sciences Lab, Kennedy Space Center, FL 32899; email: schuerg@ufl.edu.

Introduction: Robotic and piloted spacecraft are launched from Earth with finite levels of microbial contamination that are composed of species similar to the cleanroom environments within which the vehicles are assembled. After entering the harsh environment of interplanetary space, the microorganisms on the vented surfaces of spacecraft are subjected to biocidal factors that immediately begin to reduce the viable bioloads and species diversity of the launched vehicles. Based on published literature [see reviews by 1,2,3,4], between 50-70% of spore-forming bacteria, and up to 2 logs of non-spore forming species, may be inactivated during the 6-8 month cruise phase to Mars due to the solar UV irradiation (external surfaces), low pressure, and high desiccating conditions in interplanetary space.

The harsh conditions found on the surface of Mars are only slightly more conducive to the survival of terrestrial microorganisms than are found in interplanetary space. Even the launched bioloads found within pressurized human habitats in Earth-Mars transit vehicles will be exposed to conditions that are likely to reduce the species diversity of microorganisms within the human life support systems [2]. The reductions in biomass and species diversity of the launched bioloads on and within spacecraft are likely to simplify the forward contamination issues related to human expeditions on Mars by limiting the numbers of viable cells that might be dislodged from spacecraft surfaces and dispersed onto the martian terrain.

The objectives of the current study are to characterize the UV flux on Mars, predict microbial survival under martian conditions, and model the likelihood of microbial contamination of the local terrain during future crewed missions.

Methods: Experiments have been conducted under simulated martian conditions using a Mars Simulation Chamber (MSC) [described in 5] to determine the effects of solar UV irradiation, low pressure, gas composition, and low temperature on the survival of diverse *Bacillus* spp. The MSC system can accurately simulate five key components of the surface environment of Mars including: (a) pressures down to 0.1 mb, (b) UVC, UVB, and UVA irradiation from 190 to 400 nm, (c) dust loading in the atmosphere from optical depths of 0.1 (low-dust sky) to 3.5 (global dust storm) using a series of neutral density filters, (d) temperatures from –100 to 30 °C, and (e) atmospheric mixtures composed of the top five gases in the martian atmosphere [CO_2 (95.53%), N_2 (2.7%), Ar (1.6%), O_2 (0.13%) and H_2O (0.03%).

Results: The UV flux on equatorial Mars has been modeled by several teams [e.g., 1,6,7] and yields approximate fluence rates for UVA (400-320 nm) , UVB (320-280 nm), and UVC (280-200 nm) of 38, 8, and 3 W/m^2 at the mean orbital distance from the sun. These fluence rates are then decreased or increased by ~18% at aphelion and perihelion, respectiveluy, during the martian orbit. The 7 mbar atmosphere of Mars fully attenuates the UV photons below 190 nm due to absorption by the CO_2 atmosphere [8]. Thus, strong biocidal UVC irradiation is present at the marian surface.

The martian UV flux was listed by Schuerger et al. [9] as the strongest of 17 biocidal factors on Mars that include: (1) solar UV irradiation, (2) extreme desiccation, (3) low pressure (1-14 mbar), (4) anoxic CO_2 atmosphere, (5) extremely low temperatures (global average of -61 °C), (6) solar particle events, (7) galactic cosmic rays, (8) UV-glow discharges from blowing dust, (9) solar UV-induced volatile oxidants (e.g., O_2^-, O^-, H_2O_2, NOx, O_3), (10) globally distributed oxidizing soils, (11) extremely high salt levels (e.g., $MgCl_2$, NaCl, $FeSO_4$, and $MgSO_4$) in surficial soils at some sites on Mars, (12) high concentrations of heavy metals in martian soils, (13) likely acidic conditions in martian regolith, (14) perchlorates in at least some soils, (15) lack of defined energy sources free of UV irradiation, (16) no known source of available nitrogen and carbon, and (17) no obvious redox couples for microbial metabolism. These biocidal factors are consistent with other studies [10,11] that modeled conditions likely to be inhibitory to the growth of terrestrial life on Mars.

Mars chamber results and modeling [1,12,13] suggest that sun-exposed surfaces of spacecraft will be sterilized within a few tens-of-minutes to several hours on the first sol on Mars if the vehicles land under normal clear-sky conditions (optical depths < 0.5). Pressure was found to have a minor effect, and gas composition and temperature were found to have no effect on spore survival under simulated martian conditions [1]. In one example (Fig. 1), the survivability between a UV-resistant bacterium (*Bacillus pumilus* SAFR-032) and a UV-sensitive bacterium (*B. subtilis* 42HS1) exposed to a simulated Mars-normal equatorial UV flux indicated that most *Bacillus* spp. on sun-exposed surfaces are likely to be inactivated by greater than 6 orders-of magnitude within 180 min on sol 1 after landing [12]. Surface contaminants on the undersides of rovers and landers are also likely to be quickly inactivated by solar UV due to reflected UV photons off of the surrounding terrain, but the process is approximate-

ly 10-15 times slower due to the low (~3%) UV reflectivity of the regolith [13]. Furthermore, UV penetration into surface defects on spacecraft materials has been modeled (Figs. 2 and 3) [14], and results suggest that even with embedded spores, UV photons (arrows in Figs. 2 and 3) will reach the microbial cells leading to the eventual accumulation of a lethal UV dose. The only conditions in which UV irradiation cannot act on the landed bioloads are conditions in which the microrbial cells are in fully contained internal components of a rover (e.g., the computer CPU, internal payloads), are covered by UV-attenuating materials, or in which multi-layered microbial aggregates form protective layers over embedded cells [14].

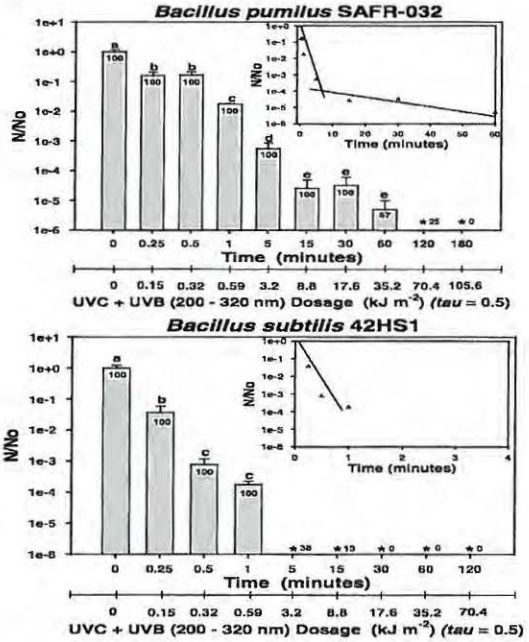

Fig. 1. Both the UV-resistant *B. pumilus* SAFR-032 (top) and UV-sensitive *B. subtilis* 42HS1 (bottom) strains were inactivated within 180 min under an equatorial Mars-normal UV flux (adapted from Schuerger et al. [12]).

Fig. 2. UV photons (arrows) can reach microbial cells (black ovals) embedded within pits, cracks, and defects on spacecraft materials (adapted from Schuerger et al. [16])

Fig. 3. UV can be attneuated by multi-layers of cells or by UV absorbing dusts, greases, etc. (adapted from Schuerger et al. [16]).

Discussion: Results suggest that a portion of the launched bioloads on spacecraft will be inactivated by the interplanetary environment before reaching Mars, a portion will be inactivated on sun-exposed surfaces of landed spacecraft within a few hours on sol 1, and survivors that are shielded from solar UV irradiation but exposed to the low pressure and low temperature of Mars may have significant difficulties growing under the environmental conditions found on the surface. However, Schuerger and colleagues [9, 14, 15] have demonstrated that at least seven genera of bacteria have members that can grow under martian conditions of 7 mbar, 0 °C, and CO_2-enriched anoxic atmospheres. Thus, we must remain cautious in concluding that the combination of 17 biocidal factors are alone capable of preventing the forward contamination of Mars.

Knowledge Gaps. The following are examples of planetary protection knowledge gaps that could be addressed with future ground and ISS research. (1) Can spacecraft coatings be designed that will decrease the aggregation of multi-layered microbial colonies during prelaunch processessing, and thus, enhance the UV biocidal effects on Mars? (2) Can spacesuits be designed that mitigate the adhesion of fine-grained surficaial fines in order to minimize the shielding effects of solar UV irradiation? (3) What is the difference between human spacesuit/habitat venting versus outgassing, and can viable cells be released by either processes? (4) How do microbial ecosystems change within human habitats over time, and can protocols be implemented that mititgate the survival of terrestrial microorganisms that might be released to the martian environment during EVAs? And (5), biocidal kill curves under martain conditions are required for a much wider diversity of terrestrial microorganisms than Bacillus spp. in order to accurately model the survival, growth, and adaption of the microbes on Mars?

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***GEODERMATOPHILUS* SP. STRAIN MN04-01 SURVIVES HIGH DOSES OF SIMULATED PRESENT-DAY MARTIAN UV RADIATION.** I. G. Paulino-Lima[1], G. G. Araujo[2], F. Rodrigues[3], E. P. Silva[4], L. J. Rothschild[5] and D. Galante[6]. [1]NPP Fellow at NASA Ames Research Center, Moffett Field, CA, USA, 94035-0001, [2]Interunities Biotechnology Graduate Program, University of São Paulo, Brazil, 05508-900, [3]Institute of Chemistry, University of São Paulo, Brazil, 05508-000, [4]Institute of Chemistry, University of São Paulo, Brazil, 05508-000, [5]NASA Ames Research Center, Moffett Field, CA, USA, 94035-0001, [6]Brazilian Synchrotron Light Laboratory, Campinas, Brazil, 13083-100.

Introduction: The ultraviolet radiation present during daytime on the surface of Mars is highly damaging for most forms of life if unprotected and totally exposed [1]. Even *Deinococcus radiodurans*, a microbial model for radiation-resistance studies, would be effectively inactivated if totally exposed to Martian UV [2], [3]. However, there might be other organisms yet to be discovered, that would not be inactivated so easily by Martian UV radiation if deposited in a clean surface such as a spacecraft. *Geodermatophilus* sp. strain MN04-01, was recently isolated from a manganese deposit in the Sonoran Desert after a screening method developed for selecting highly UV-resistant microorganisms. This isolate is 3 times more resistant to UV-C radiation than *D. radiodurans*, as measured through colony counts on agar plates. Here we report its survival to a simulated Martian UV irradiation experiment performed at NAP-Astrobio, University of São Paulo, Brazil.

Material and Methods:

Microbial cultures: Cells of *Geodermatophilus* sp. strain MN04-01 were grown in GOM Medium (1.5% Malt Extract, 1% starch, 1% sucrose, 0.5% yeast extract , 0.2% $CaCO_3$) for 7 days at 30 °C, 200 rpm. Cells of *Deinococcus radiodurans* were grown in TGY medium (1% tryptone, 0.6% yeast extract, 0.2% glucose) for 15h at 30 °C, 200 rpm. 0.5 ml aliquots of each organism were centrifuged at 8,000 rpm for 3 min and the cell pellet was washed twice through centrifugation in the same conditions using saline solution (0.9% NaCl). Finally, the cells were resuspended in 0.5 ml of saline solution.

Sample loading: Four replicates of 2 µl aliquots of the cell suspension were loaded in four 5 mm x 5 mm silicon support (Si 111). After 10 min of dehydration in a laminar flow hood at room temperature the silicon supports were fixed on a metallic mount using a carbon tape. The mount containing the samples was then fixed inside the Mars Simulation Chamber.

Experimental conditions: The Martian uv flux was simulated using a non-ozone free Oriel Solar Simulator containing a xenon-arc lamp emmiting a broad spectrum of UV, visible and infrared radiation. To minimize the amount of infrared radiation delivered, an air mass 0 (AM0) filter was used to correct the lamp spectrum and a water filter was placed between the solar simulator and the vacuum chamber (Fig. 1). The uv flux was measured usin a Vilber Lourmat radiometer as 87 W/m^2 for UV-A, 118 W/m^2 for UV-B and 23 W/m^2 for UV-C.

The samples were irradiated at room temperature under an atmosphere of 8 mbar containing 95% CO_2 and 5% N_2, to the following Martian full uv doses, in kJ/m^2: 3, 6, 10, 30, 60 and 100.

Analytical techniques: After the irradiation, individual silicon supports containing the samples were placed in microfuge tubes containing 100 µl of appropriate culture media and vortexed for at least 20 seconds. Cell suspensions were serial diluted 10^{-1} to 10^{-4} and 10 µl aliquots were inoculated on agar plates, which were incubated at 30 °C for up to 10 days. Colonies of irradiated samples (N) and the non-irradiated control (N_0) were counted and the results (N/N_0) were plotted in a graph showing survival curves. The remaining volumes of the 10^{-1} dilutions were also stained with propidium iodide and SYTOX® green for live/dead quantification through fluorescence microscopic analysis.

Fig. 1. Experimental setup showing: solar simulator (S), water filter (W), AM0 filter (F), radiometer (R), vaccum chamber (V), and biological specimens being irradiated (B).

Results:

Colony counts: LD_{10} (dose that kills 90% of the population) was 3.27 ± 0.25 kJ/m^2 for *D. radiodurans* and >100 kJ/m^2 (maximum dose tested in our experiments) for *Geodermatophilus* sp. strain MN04-01 (Fig. 2).

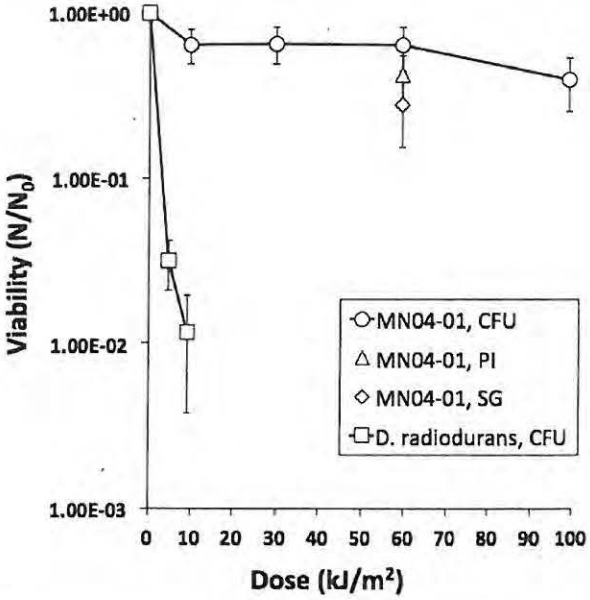

Fig. 2. Survival of *Geodermatophilus* sp. strain MN04-01 to full uv (200-400 nm) under martian atmosphere, and comparison with *D. radiodurans*. Fluorescence microscopy data is also shown on the graph. CFU, colony forming-units. PI, propidium iodide. SG, SYTOX® green.

Fluorescence: After analyzing 13 micrographs for the non-irradiated control and 14 for samples irradiated with 60 kJ/m^2 of Martian UV irradiation (Fig. 3), both staining methods resulted in similar numbers (Fig. 4).

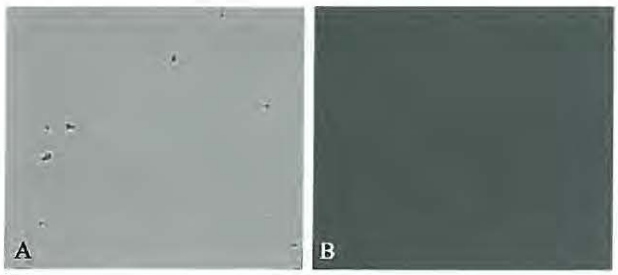

Fig. 3. A, Phase contrast micrograph showing cells of *Geodermatophilus* sp. strain MN04-01. B, Fluorescence micrograph (SYTOX® green) showing dead cells of the same organism after 60 kJ/m^2 of Martian full uv irradiation.

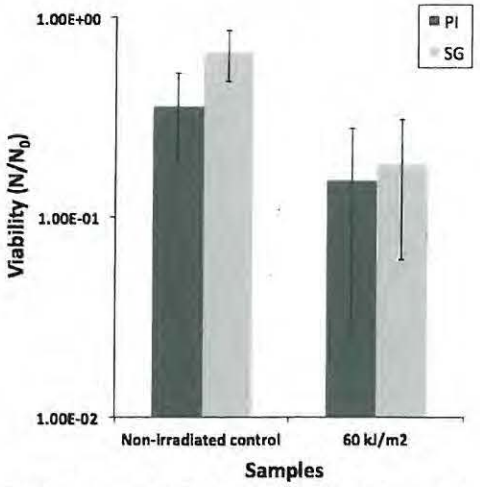

Fig. 4. Viability of strain MN04-01 to 60 kJ/m^2 of Martian full uv irradiation as determined by fluorescence microscopy using propidium iodide (PI) and SYTOX® green (SG).

Raman spectroscopy of colonies (Fig. 5) indicates the production of a melanin-like pigment that strongly absorbs UV-C radiation.

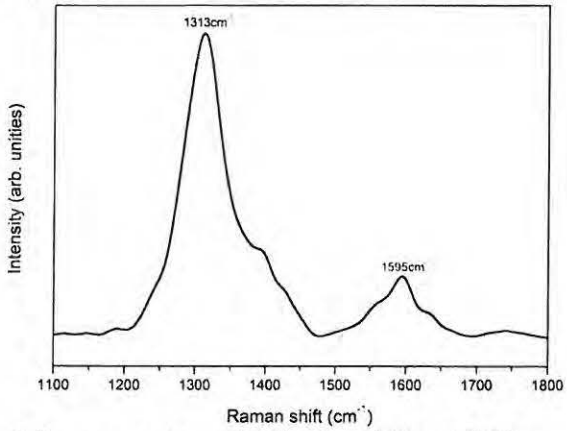

Fig. 5. Raman spectrum showing two shifts, at 1313 cm^{-1} and 1595 cm^{-1}, consistent with melanin.

Conclusions: The results obtained in this research have implications for planetary protection and space exploration using biological systems. This microbial isolate represents an excellent biological model for photobiology studies including DNA damage and repair analysis.

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PREDICTING THE RESPONSE OF TERRESTRIAL CONTAMINATION ON MARS WTH BALLOON EXPERIMENTS IN EARTH'S STRATOSPHERE. D. J. Smith[1] and E-MIST Team[2], [1]NASA Ames Research Center, Mail Code: SCR, Space Biosciences Research Branch, david.j.smith-3@nasa.gov [2]NASA Kennedy Space Center, Engineering and Technology Directorate

Introduction: We designed, built and flew a self-contained payload, Exposing Microorganisms in the Stratosphere (E-MIST), on a large scientific balloon launched from New Mexico on 24 Aug 2014 [1]. The payload carried *Bacillus pumilus* SAFR-032, a highly-resilient spore-forming bacterial strain originally isolated from a NASA spacecraft assembly facility. Our balloon test flight evaluated microbiological procedures and overall performance of the novel payload. Measuring the endurance of spacecraft-associated microbes at extreme altitudes may help predict their response on the surface of Mars since the upper atmosphere also exerts a harsh combination of stresses on microbes (e.g., lower pressure, higher irradiation, desiccation and oxidation) [2].

Materials and Methods: Our payload (83.3 cm x 53.3 cm x 25.4 cm; mass 36 kg) mounted onto the exterior of a high altitude balloon gondola. Four independent "skewers" rotated 180° to expose samples to the stratosphere. During ascent or descent, the samples remained enclosed within dark cylinders at ~25 °C. Each skewer had a base plate holding ten separate aluminum coupons with *Bacillus pumilus* spores deposited on the surface. Before and after the flight, *B. pumilus* was sporulated, enumerated and harvested using previously described techniques [3–5].

Major payload components were a lithium-ion battery, an ultraviolet (UV) radiometer (400 to 230 nm), humidity and temperatures sensors, and a flight computer. During the test flight, samples remained in a sealed position until the payload reached the lower stratosphere (~ 20 km above sea level). Next, the flight computer rotated the skewers into the outside air. After a short rotation demonstration (2 seconds), all skewers reverted to the closed position for the remainder of the flight. The payload continued floating at an altitude of 37.6 km for 4 hours before beginning a 23 minute descent on parachute.

Results and Discussion: Our first test flight examined unknowns associated with sample transportation, gondola installation, balloon ascent/descent, and time lingering in the New Mexico desert awaiting payload launch and recovery. We created a batch of experimental control coupons (each containing approximately 1 x 10^6 spores) used throughout the investigation for ground and flight test purposes. Several treatment categories were evaluated: Lab Ground Coupons (kept in the KSC laboratory); Transported Ground Coupons (traveled to New Mexico and back but not installed in payload); and Flight Coupons (flown). A subset of coupons from each treatment category were processed, resulting in statistically equivalent viability (Kruskal–Wallis rank-sum test at a 95% confidence level). Taken together, nearly identical viability from all coupons indicate that balloon flight operations and payload procedures did not influence spore survival. A negative control (blank, sterile coupon) was also flown to verify payload seals prevented outside contamination.

A species-specific inactivation model that predicts the persistence of microbes on the surface of Mars is one of many possible outcomes from balloon experiments in the stratosphere. The simplicity of the payload design lends itself to customization. Future investigators can easily reconfigure the sample base plate to accommodate other categories of microorganisms or molecules relevant to the Planetary Protection community. If future flights exposed microbes for hours, we would expect to see a rapid inactivation. Smith et al. [6] simulated stratospheric conditions and measured a 99.9% loss of viable *Bacillus subtilis* spores after only 6 hours of direct UV irradiation. Earth's stratosphere is extremely dry, cold, irradiated, and hypobaric, and it may be useful for microorganisms isolated from NASA spacecraft assembly facilities to be evaluated in this accessible and robust Mars analog environment.

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March 2015

LOW DISPERSAL OF HUMAN-ASSOCIATED MICROBES ON TO PRISTINE SNOW DURING AN ARCTIC TRAVERSE ON SEA ICE BY THE *MOON-1* PLANETARY SURFACE ROVER. A. C. Schuerger[1] and P. Lee[2,3,4], [1]University of Florida, Space Life Sciences Lab, Kennedy Space Center, FL 32899; email: schuerg@ufl.edu; [2]Mars Institute, [3]SETI Institute, [4]NASA Ames Research Center, MS 245-3, Moffett field, CA 94035; email: pascal.lee@marsinstitute.net.

Introduction: Future human missions to Mars will transport terrestrial microorganisms to the sites of exploration. This will be an unintended experiment in directed panspermia [1]. In April 2009, a modified Humvee vehicle called the *Moon-1* rover (Fig. 1, background) conducted a 496 km traverse over sea ice along the Northwest Passage from Kugluktuk to Cambridge Bay, Nunavut, Canada. This *Northwest Passage Drive Expedition* was carried out under the auspices of the NASA Haughton-Mars Project in support of field studies of pressurized rovers in future Moon/Mars human exploration. During the traverse, team members collected samples from within the *Moon-1* rover, and from pristine snow-covered surfaces around the rover at three overnight locations (Fig. 1, foreground). The objective was to determine the extent of microbial dispersal away from the *Moon-1* rover and on to pristine snow during EVA activities.

Methods: The *Moon-1* rover is a non-pressurized all-terrain rover simulating some of the basic attributes of a pressurized planetary rover, including the ability to traverse unprepared terrain and offer shelter to a crew of five. The diesel-powered *Moon-1* was accompanied by two gasoline-powered snowmobiles, with each snowmobile pulling a *komatik* sled loaded with food, fuel, and other equipment and supplies.

Samples of surface snow were collected in sterile 50 cc plastic tubes at three sites along the traverse: Sites A, B, and C. At each of these sites, six tubes of samples were collected systematically in specific locations in relation to the Humvee (Fig. 2): 1 = inside, on driver side floor; 2 = inside, in drainage gutter on rear access steps; 3 = outside, upwind; 4 = outside, downwind; 5 = outside, up-track; 6 = outside, down-track. Site A was a brief science stop during day 1 of the 7-day traverse. Sites B and C were overnight stops with samples collected in the morning. Food was prepared and consumed outside the Humvee within 3 m of the rear of the vehicle at Sites B and C.

Microbial characterization. Snow and ice samples were kept frozen (-25 to -5 °C; HOBO data logger) during the traverse, and then shipped on-ice to microbiological labs at Kennedy Space Center, FL for processing (Schuerger lab). After melting snow samples at 4 °C for 48 hrs, aliquots of all samples were plated on R2A agar plates at undiluted rates. Samples were incubated at 37, 24, or 4 °C, and the numbers of colony-forming units (cfu's) per sample and temperature were estimated after 7 and 28 days of incubation. Unique colonial morphotypes were recovered and purified from all petri plates; over 200 individual isolates of bacteria and fungi were collected and archived.

Fig. 1: Microbial sampling of pristine snow in the vicinity of the *Moon-1 Humvee Rover* (background).

Fig. 2: *Moon-1* sampling pattern for human associated microorganisms.

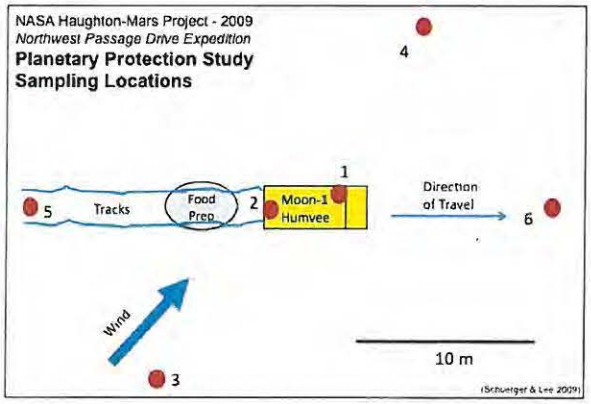

Table 1: *Moon-1* traverse summary data.

Recovered isolates of culturable bacteria and fungi were processed with established 16S and 18S sequencing protocols [2,3,4,5]. 16S and 18S sequences were then generated by the Interdisciplinary Center for Biotechnology Research (ICBR) at the University of Florida. All sequences were subsequently identified using the BLAST library on the NCBI website (http://blast.ncbi.nlm.nih. gov/Blast.cgi).

Results: All samples collected from within the *Moon-1* rover were heavily populated by culturable

bacterial and fungal species. Bacterial populations in samples collected within the *Moon-1* rover ranged between 1.25 x 10^2 and 2.7 x 10^3 cfu/mL for samples incubated at 37 °C, and from 10 to 1.1 x 10^3 cfu/ml for cultures incubated at 24 and 4 °C. Fungal populations from inside the rover ranged between 2-3 cfu/mL and 2.5 x 10^2 cfu/mL. All snow samples from the upwind, downwind, up-track, and down-track sample sites exterior to the *Moon-1* rover were negative for both bacteria and fungi except for two cfu's recovered at Site A from sample A4 (downwind; bacterium) and A5 (uptrack; fungus). The bacterial isolate was identified as *Brevibacillus agri* (accession JX517278; closest match 0.998), and the fungal isolate was identified as *Aspergillus fumigatus* (accession JX517279; closest match 0.991). Both microbes were also recovered from within the *Moon-1* rover. Thus, while the internal samples from the *Moon-1* rover contained a wide range of colony morphotypes of culturable bacteria and fungi, only two individual colonies (1 bacterial and 1 fungal) were recovered from all of the snow samples collected exterior to the rover.

Fig. 3: *Top row*:Microbial diversity typically observed for samples collected from within the *Moon-1* rover. *Bottom row*: Most snow samples from outside the rover were negative for culturable bacteria and fungi.

The dominant fungi recovered from within the *Moon-1* rover include species from the genera *Alternaria*, *Aspergillus*, *Cladosporium*, *Geomyces*, *Phoma*, *Penicillium*, and *Tetracladium*. The dominant bacterial species recovered from within the *Moon-1* rover were *Bacillus circulans*, *B. licheniformis*, *B. megaterium*, *B. psychrodurans*, *B. subtilis*, *B. simplex*, *Brevibacillus borstelensis*, *Kocuria rosea*, *Microbacterium paraoxydans*, *Paenibacillus pabuli*, *P. illinosensis*, *P. amlolyticus*, and *Sporosarcina aquimarina*.

Discussion: The results support the conclusion that human-associated microorganisms were not easily dispersed on to the snowy terrains during the *Moon-1* traverse. The sampling was designed to investigate contamination of pristine snow aound the *Moon-1* vehicle as a possible prediction of airborne microbial dispersal away from a crewed rover and EVA activities by humans during a future crewed Mars mission. The recovery of only one bacterium and one fungus on hundreds of R2A petri plates from 11 snow samples is also consistent with the emplacement of the microbes in to the samples during the collection process in the field or lab contaminantion during processing. Current data did not permit the elimination of these latter two possibilities. But clearly, snow contamination of the snow sample sites along the 496 km traverse of the *Moon-1* rover did not occur at a high rate of exchange between the crewed rover and the local terrain.

The implications for a human mission to Mars is that even if crew members are involved in collecting field samples, as long as they are using sterilized implements when interacting with the terrain, they are likely not to contaminate sample sites. Furthermore, two additional factors present on Mars, but lacking in the Arctic, are likely to reduce the chances of contaminating regolith or rock samples during human missions: (1) all humans will be fully contained within sealed spacesuits which will greatly isolate their indigenous microflora during EVA activities, and (2) the surface of Mars has at least 17 biocidal factors [6] that will increase the inactivation of human-associated microbes on the outside surfaces of spacesuits, equipment, and rovers; thus reducing viable external bioloads during human missions on Mars. It is likely that the biocidal stresses on Mars will be several orders of magnitude harsher than were encountered during the *Moon-1* mission, and thus, potential microbial dispersal is likely to be reduced proportionally from that described herein.

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Near space biological research using weather balloons

By Samuel Mark Harrison

Address - 1 5 Lucerne Street San Francisco California 94103
Email - samharrison720@gmail.com

Introduction:

On the 11th of January 2015 myself (Samuel Mark Harrison) a Bournemouth University Student and Daniel Parker both from the UK launched our Shackleton 2 near Space Probe (pictured). This was part of project to test ultra low-cost near-space vehicles that can be used to carry out near-space research. Our probe travelled over 300 miles (flight path pictured) and endured the hostile environment of near space. It experienced temperatures as low as -50 degrees Celsius, a near vacuum at its peak attitude of 89,000ft and speeds of over 150mph. Our probe was carried to this altitude using a 2000g PAWAN Indian weather balloon filled with helium.

By using these near space probes research can be carried out into planetary protection by launching biological samples into the extreme conditions that space offers. This allows us to see which types of sample biological contamination thrive even in near space. This represents a small but vital step to ensuring we don't contaminate future planets particularly in the context of manned missions when the risk of biological contamination is far greater. Due to the levels of altitude which weather balloons can operate at (up to 130,000ft) research can also be carried out into the effects of cosmic radiation on cells. This would be of great relevance both in the context of contamination but also on the ability for humans to survive outside of earth's environment.

Basic probes complete with tracking system can be launched and recovered for a fraction of the cost of other alternatives which allows this area of research to be far more accessible for students on a global level. Given the rise of nations such as India and China and their respective space programs this technology is a fantastic low-cost tool for building the skills of future bright minds around the world for the manned missions of the future.

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